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# THE CLINICAL AND PATHOLOGIC EFFECTS OF THE NITROGEN AND SULFUR MUSTARDS IN LABORATORY ANIMALS*

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The sulfur (bis-β-chloroethyl sulfide) and nitrogen mustards (alkyl secondary and tertiary β-chloroethyl amines) have been investigated for their pharmacologic action, toxicity, mechanism of action, clinicopathologic effects, and the therapy of their effects. Other reports [1-4] have dealt or will deal with their pharmacology, toxicity, and certain physiologic and therapeutic aspects. This report is concerned chiefly with their clinical and pathologic effects. Due to restrictions imposed by wartime security requirements, many of the data available in classified documents of other American, Canadian, and British investigators can be cited only on the basis of the original reports which are not yet available in the open literature. Where priority of observations is concerned, these reports are cited as dated personal communications.

Attention was focused in our work on the clinical and pathologic effects of the following compounds (designated by the official War Department symbols for brevity); the essential toxicity data are taken from Anslow, Karnofsky, Jager, and Smith.[1,2]

H = bis(β-chloroethyl) sulfide

$HN_1$ = ethyl-bis(β-chloroethyl) amine

$HN_2$ = methyl-bis(β-chloroethyl) amine

$HN_3$ = tris(β-chloroethyl) amine

TL 301 = iso propyl-bis(β-chloroethyl) amine

Since our most complete studies were made on $HN_2$, methyl-bis (β-chloroethyl) amine, the results are reported in detail for this compound as the prototype for these vesicants. Where deviations occurred in identical tests with the other agents, the illustrative data are included.

There is ample evidence that the nitrogen compounds resemble H in

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their biologic effects. The free bases are vesicants capable of injuring all tissues with which they come in contact in sufficient dosage. Their systemic effects are notably similar in time relations and in the selected tissues that are most distinctively affected.

## SECTION I

### CLINICAL EFFECTS

The original observations reported herein have been confined principally to rodents (albino mice, albino rats, and New Zealand rabbits obtained from Carworth Farms) and some dogs. Where significant data are available, reference is made to the effects of the compound in other species.

#### *Influence of Route of Administration*

The *subcutaneous, intravenous, intramuscular,* and *intraperitoneal* routes of administration provide accurate modes of giving measured dosages of candidate compounds for toxicologic appraisal. When volatile substances are tested *percutaneously,* the loss of agent, by evaporation or local fixation, introduces considerable variation when correlating dosage with systemic effects. Obviously, changes in environmental temperature and humidity affect vaporization through their influence on skin temperature. Rates of absorption are also influenced by skin temperature, moisture, and the rate of penetration of each compound.[5]

The appraisal of mortality and clinicopathologic effects *via inhalation* of the vapor, as in the case of $HN_1$, $HN_2$, and H, is especially difficult because of the unknown quantity of inhaled dose. In the case of a nonvolatile agent dispersed as a particulate (as has been necessary with $HN_3$ until very recently) the measure of concentration in a gassing chamber as a point of reference for the inhaled dose is especially dubious. It is probably better than none, but one should not overlook the factors that may promote or retard inhalation and absorption of a particulate, *viz.*, particle size, the degree of impaction and arrest in the nose, variations in respiratory rate and volume, absorption from the nose, species variations, and, particularly, impaction on the body fur necessitating efforts or devices to prevent licking and oral ingestion of agent from contaminated fur.

It is important to note that the $LD_{50}$ is an arbitrary statistical value, encompassing a wide, often unaccounted for, range of variation and even different modes of death. At the $LD_{50}$, 5 of 10 intoxicated animals may be expected to die; at 0.5 $LD_{50}$ only one of 10 may be lethally intoxicated; yet the 5 animals dying at the $LD_{50}$ and the single animal dying at 0.5 $LD_{50}$ may show the same degree of pathologic injury.

Similarly, within the 10 animals receiving an $LD_{50}$ dose, the pathologic changes may range from almost nil to the most extreme. Hence the actual dose administered may sometimes be of little value in estimating biologic effect; each animal must be appraised individually on the basis of its particular symptoms and pathologic changes.

The form of the compound had to be varied for certain routes of administration. For gassing and cutaneous experiments the free bases were used; for intravenous and subcutaneous injections the HCl salts of the nitrogen mustards were employed. We have assumed, without direct proof, that the injection of the HCl salts was equivalent to the injection of the free base.

The problems presented by the intravenous dosage of H have been especially vexing. The literature from World War I to date on the toxicity of H is inconsistent. This is not surprising when one realizes the difficulties of administering this compound intravenously. After gassing or skin application the results are fairly consistent, if licking or swallowing of H is avoided. The subcutaneous route also yields a uniform $LD_{50}$ and pattern of systemic effects. But the intravenous route has been unpredictable. We have observed considerable variation in the $LD_{50}$ of H via this route which we believe to be due to the mode of administration. When H is given neat and slowly, the $LD_{50}$ for rabbits is about 4.8 mg. per kg., but when given rapidly it is 3.6 mg. per kg. When given in propylene glycol so that the volume of the solvent is 0.5 cc. per kg. of rabbit the $LD_{50}$ falls to 2.7 mg. per kg. When given neat, H lodges in the pulmonary capillaries, and an indefinite amount reacts locally and is not available to produce systemic injury. Neat H is immiscible with the blood and when given intravenously the droplets act like fat emboli and are distributed according to size, rate, and number of droplets introduced. Pulmonary injury going on to infarction and necrosis was found even after the administration of supra $LD_{50}$ doses of H dissolved in propylene glycol. Earlier workers [6-10] also have reported pulmonary injury after H but did not relate it to the toxicity of the compound or account for its pathogenesis. To avoid pulmonary injury by H droplet-embolization we have administered the agent dissolved in propylene glycol, and at dosages in which toxicity was least likely to be affected by pulmonary deposits.

To avoid comparisons with animals dying from acute neurologic injury (within 8 hours or less), or from late complications (after 5 days), most of the animals were killed at the point of death or sacrificed at 72 or 96 hours in the comparative study of pathologic effects. A few animals examined at 48 to 120 hours are included in some of the tabulations.

*Individual Pharmacologic Actions*

For general interest, a summary of the individual pharmacologic actions is given but the reader may consult Gilman and Phillips [11] for further comments. Above $LD_{50}$ doses, certain characteristic pharmacologic actions are manifest chiefly on the nervous system. They are readily elicited on intravenous administration, less readily on administration by other routes.

*Cholinergic action* is possessed by both H and $HN_2$, and is characterized by "muscarinic" action on glands and smooth muscle and by "nicotinic" action on autonomic ganglia and skeletal muscle. In the case of both agents, peripheral stimulation appears to account for the "muscarinic" responses, although central excitation, apparently minimal, cannot be excluded. Cholinergic action is not considered a factor in the development of the syndrome leading to delayed death, and *is not responsible for late vomiting and diarrhea.* Whether or not cholinesterase inactivation is involved in this cholinergic action has not been determined.

*Parasympathicolytic action* is shown by $HN_3$ at small doses, as judged by paralysis of cardiac vagal fibers and antagonism of the action of parasympathomimetic drugs. A similar parasympathicolytic action of H, $HN_2$, and TL 301 appears to be present in severe intoxication.

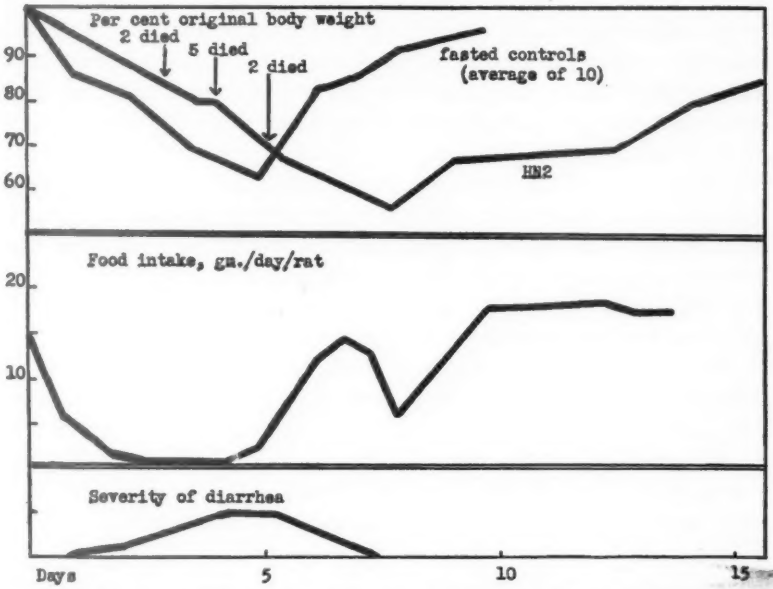
*Convulsive action* is so distinctive of $HN_3$ as to warrant classification of this agent as a true convulsive drug. High doses of this compound given parenterally have an immediate, direct, intense convulsive action which results in death within 6 hours. Central stimulation is a property of H and $HN_2$ at high doses, but the action is not similar to that of $HN_3$.

*Paralytic action* is possessed by both $HN_2$ and $HN_3$. This action is characterized by a progressive, irreversible muscular weakness and may cause death in a number of hours.

*Neurologic injury* has been observed in animals intoxicated with $HN_1$ and $HN_2$ by either gassing or intravenous administration, but never after intoxication by other routes or with the other agents. This injury has usually appeared about the third or fourth day in animals showing no other evidence of systemic intoxication. In extreme cases, the injury is fatal, but among survivors hyperirritability remains for weeks.

*Neurologic Death.* Large doses given parenterally (5 to 10 $LD_{50}$ or more) induced neurologic injury and death in a few hours, the outstanding manifestations of intoxication consisting of convulsions, depression, loss of coordination, irritability, tremors, weakness, dyspnea,

evidence of parasympathomimetic activity—salivation, lacrimation with red tears in rats, defecation, and urination—and terminal respiratory paralysis. At the lower doses producing neurologic effects, animals survive up to 40 hours and also show evidence of delayed systemic intoxication. (Intravenous injection induces neurologic signs at lower doses, relative to the $LD_{50}$, than subcutaneous injection.)




Text-Figure 1. Data on 10 rats receiving 1.7 mg. per kg. of $HN_2$ intravenously (1.5 $LD_{50}$).

*Delayed Death from Systemic Intoxication.* Mice, rats, rabbits, and dogs, receiving 1 to 3 $LD_{50}$ parenterally, may remain relatively asymptomatic for 24 to 48 hours, except for mild anorexia and slight weight loss. Following this latent period debilitation is rapid (Text-Fig. 1). The fur becomes ruffled and the animal huddles in a corner; it stops eating and drinking and becomes progressively weaker, although somewhat hyperirritable, and develops watery diarrhea. The body temperature falls and there is rapid decrease in body weight, partly attributed to the loss of fluid through the intestinal tract. The animal becomes progressively more depressed, apathetic, and unresponsive as death approaches. In rabbits the symptoms of intoxication are not as marked; and although eating and drinking stop and mild diarrhea develops, weight loss is not as severe and the animal remains

fairly responsive until shortly before death. Severe leukopenia is present in all four species at death. A dog receiving 2.5 mg. per kg. of $HN_2$ intravenously was relatively asymptomatic until just prior to death on the fourth day. About 5 mg. per kg. of $HN_2$ in dogs was fatal in 2 to 4 days, inducing vomiting, incoordination, bloody diarrhea, repeated convulsions, and anorexia prior to death.

At autopsy, intoxicated animals appear emaciated and dehydrated. The stomach is distended with food and fluid, and the small intestine, especially the duodenum, is distended with yellowish fluid which is occasionally mucoid in nature, and the intestinal wall is transparent and friable. Small hemorrhagic and hyperemic areas may occur in the wall of the small intestine, and ulcerations of the mucosal surface of the distal part of the ileum are noted occasionally. In rats, Peyer's patches appear hyperemic and the cecal lymph nodes are frequently hemorrhagic. The cecum is usually filled with food and fluid, and the colon is often empty or contains mucus, gas, or fluid feces (notably in rabbits). In rats the adrenal glands are markedly enlarged. In dogs, the intestinal lesions are complicated by hemorrhagic ulcers.

The spleen is small and comparatively bloodless, the thymus is atrophic, and the lymph nodes sometimes appear hemorrhagic or are so greatly reduced that they are difficult to identify. The bone marrow is either red and liquid, or fatty in appearance.

Fowler and Grant [12] have described pulmonary edema in guinea-pigs after subcutaneous, but not after intraperitoneal, injection of $HN_2$ hydrochloride.

This clinicopathologic picture is designated in this report as *delayed death* from systemic intoxication, and is to be distinguished from deaths occurring 7 days or longer after intoxication, the specificity of which is in question, and which we designate as *remote deaths*.

*Remote Deaths.* While most mice and rats receiving $LD_{50}$ doses parenterally and surviving 7 days effect a complete recovery, in a few weight loss continues and death may occur as long as 20 days after injection. In such animals, weight loss is severe, in the range of 30 to 50 per cent of the original body weight. Hodge *et al.*[13] have shown in acute starvation that 70 per cent of 3-months-old mice die within 4 days, after an average weight loss of 30 per cent, although older mice under the same conditions may live slightly longer; while Mulinos and Pomerantz [14] have found that weight losses of 35 to 45 per cent may be fatal in rats. Most of our animals with remote deaths were in this dangerous range of weight loss and therefore death cannot be charged specifically to the late effects of $HN_2$. In many instances infection was an obvious complication, and it may be suspected that, in others, less

evident infection contributed to the persistence of anorexia and progressive debilitation. The possibility of a direct lethal action effective beyond 7 days appears unlikely.

After the crisis of intoxication which occurs between 70 and 120 hours, there ensues a rapid recovery of the leukocyte count and a slow regain of weight. Wilson, Vars, and Gurin [15] have noted that rats surviving a dose slightly greater than the $LD_{50}$ occasionally regain weight but remain extremely apprehensive and hyperirritable. The same phenomenon was sometimes noted in our rats. Mice surviving $LD_{50}$ doses are capable of bearing normal young within a few weeks after intoxication. Observations on this point have not been made in other species.

*Effects of Different Routes of Administration*

By cutaneous application of the *free base,* the $LD_{50}$ of $HN_2$ is reported as 35 mg. per kg. for mice,[16] 14 to 15 mg. per kg. for rats,[17,18] 12 mg. per kg. for rabbits,[17] 20 mg. per kg. for goats,[17] and about 50 mg. per kg. for monkeys.[18] The clinical picture, when recorded, is typical of systemic intoxication. The factors of skin thickness, temperature, local fixation, evaporation, and the rate of penetration of the applied liquid vary with the species, the site of application, and the environment, so that toxicity figures are chiefly of value for comparison with the cutaneous $LD_{50}$ of other compounds.

The $LD_{50}$ of $HN_2$ hydrochloride by subcutaneous (2.6 mg. per kg.[2]) or intraperitoneal (about 2.3 mg. per kg.[2]) injection in mice is not more than 50 per cent greater than the *intravenous* $LD_{50}$ (about 2.0 mg. per kg.[2]). The free base appears to be somewhat less toxic.[19] There are no marked differences in susceptibility among mammals.[2]

*The influence of ingested food on the oral $LD_{50}$* for $HN_2$ was shown as follows. Fed mice receiving orally an $LD_{50}$ solution of the hydrochloride (0.4 cc. of a 45 to 250 mg. per cent solution in saline) die over a period of 4 to 7 days and the symptomatic picture and changes at autopsy are typical of delayed death. The $LD_{50}$ was 20 mg. per kg. Mice receiving higher doses (50 mg. per kg.) developed severe hemorrhagic and ulcerative lesions in the duodenum and jejunum, with occasional perforations, and deaths occurred within 2 to 3 days. In fasted mice (*i. e.,* deprived of food for 18 to 24 hours before the intraesophageal administration of the hydrochloride, and then re-fed shortly afterwards) the $LD_{50}$ is 10 mg. per kg. The great majority of deaths occurred within 2 to 3 days, the animals showing at autopsy extremely severe changes in the duodenum and jejunum with hemorrhage, ulceration, and in some cases perforation. Mice surviving this period usually

recovered. It is concluded that food and gastrointestinal secretions detoxify or bind part of the $HN_2$ and thus limit local damage; this process, however, does not appear to prevent the absorption of the compound in a toxic form.

Mice and rats *gassed* at 1 to 2 $LC_{50}$ (0.3 to 0.6 mg. per liter for 10 minutes) develop dyspnea, wheezing, nasal secretion and opaqueness of the cornea, and death usually occurs 72 to 144 hours after gassing, with the picture of systemic intoxication. Rabbits have more severe respiratory symptoms, but systemic absorption of the compound, nevertheless, contributes largely to death. Remote deaths are common in animals surviving this critical period, but these are scattered in a random manner over the period of 5 to 20 days; such animals continue to lose weight and frequently show persistence of respiratory difficulties. Respiratory infection unquestionably plays an important part in these remote deaths and rats that have recovered their initial body weight may continue to show excessive nasal secretion for 3 to 4 weeks after gassing.

Mice gassed at 2 to 3 $LC_{50}$ develop severe neurologic disturbances with agitation, ataxia, and incoordination, these symptoms diminishing within 48 hours. Most of the deaths do not occur until 72 to 100 hours after gassing. Rats also show neurologic symptoms at this dose, but die within 40 hours, apparently of a combination of neurologic, respiratory, and systemic injury. Neurologic manifestations at low multiples of the $LC_{50}$, an occurrence not noted after equivalent subcutaneous doses, have also been described by the University of Chicago Toxicity Laboratory.[20]

Dogs, cats, and goats are reported to suffer from such severe respiratory injury in the way of edema of the glottis, necrosis and sloughing of the epithelium of the trachea and bronchi, with pulmonary injury (which may in some cases be secondary to infection), that they undergo an early respiratory death[20,21] rather than the delayed death (about 100 hours) associated with systemic intoxication and typical of gassed mice and rats. This may explain why the $LC_{50}$ in larger animals is significantly lower than in mice, rats, and rabbits.

The vapor causes redness of exposed surfaces and occasionally edema of the ears and male genitalia of mice, rats, and rabbits, but severe cutaneous lesions are not produced at $LC_{50}$ doses.

## Section II

### SYSTEMIC EFFECTS OF $HN_2$ AND H

Irrespective of the route by which $HN_2$ or H enters the body of mice, rats, and rabbits (percutaneously, subcutaneously, intravenously,

orally, or by gassing), they exert certain qualitatively consistent pathologic effects in doses at or moderately above the $LD_{50}$, notably affecting the blood-forming organs and small intestine. In many cases these systemic effects appear to be more important in causing death than the local injury and they will be described without specific reference to the route of administration. The susceptibility of the tissues affected was appreciated during and after World War I.[6-8,22,23] In this report we have omitted detailed consideration of the local effects of these compounds for the sake of brevity.

Routine pathologic examination has consisted of the study of the larynx, trachea, and lungs, several levels of the esophagus, stomach and intestinal tract (longitudinally rolled segments of the entire intestinal tract were prepared in mice, rats, and some rabbits; in dogs large pieces were similarly rolled to provide adequate samples), liver and gallbladder, pancreas, kidneys and urinary bladder, spleen, thymus, lymph nodes, femoral and sternal bone marrow, testes and ovaries, thyroid, adrenals, and heart muscle. Occasional specimens of the eyes and nose were studied in cross sections of the entire head.

Tissues were obtained by sacrifice or at death, and routinely fixed in Zenker's fluid, cut at 6 $\mu$, and stained with hematoxylin and eosin. Sections of the spleen and bone marrow were stained with Giemsa's stain, or eosin azure after Maximow. Sections of the marrow were cut at 3 to 4 $\mu$. Special stains for iron and fats were used occasionally.

## HEMATOLOGIC EFFECTS OF HN2

Leukopenia, the most distinctive feature of intoxication yet described, has been found in all species studied: mouse, rat, rabbit, guinea-pig, dog, cat, goat, chicken, pigeon, monkey, and man. It was first reported in gassed mice.[20] We have followed the course of hematopoietic injury in mice, rats, and rabbits by means of daily total and differential counts. The leukocyte counts were done in the usual manner, the smears being stained with Wright's or the Jenner-Giemsa stains.

### *Mice*

One hundred leukocyte counts and 75 differential counts were made on specimens of tail blood of mice before and after subcutaneous administration of the HN2. Our normal average leukocyte count (based on the examination of 52 tail bloods) was 26,150 (range, 12,250 to 49,000) per cmm. Leukocyte counts on heart blood have been reported to range from 4,000 to 6,000.[24] Differential examination was somewhat more consistent, but variations were wide. About 10 per cent of the erythrocytes showed polychromasia, an incidence recorded by others.[25]

Because of great variations in the normal tail blood, the mouse is not regarded as a satisfactory subject for routine or quantitative hematologic studies. Therefore only the unequivocal changes found in intoxicated animals are presented.

Subcutaneous administration of *30 to 40 mg. per kg.* of $HN_2$ HCl was fatal in 12 to 40 hours. Leukocytosis was present within 12 hours, but in the animals surviving longer the count had begun to fall. The leukocytosis was due entirely to an increase in granulocytes, since lymphopenia occurred early. Polychromasia was not affected. The platelets on the smear appeared to be increased in numbers, although no counts were done.

Administration of *3.0 to 8.0 mg. per kg.* subcutaneously was fatal in 72 to 144 hours. Lymphopenia with granulocytosis of variable degree appeared within 12 hours and was followed by a precipitous decline in the total leukocyte count. Four days after injection the average leukocyte count was 3,000 and polychromasia was reduced or absent.

TABLE I

*Effects of 3.0 mg. per kg. of $HN_2$ Hydrochloride Given Intravenously in Albino Rats*

| Hours after injection | No. observed | White blood cell count | Differential count | | | | |
|---|---|---|---|---|---|---|---|
| | | | Granulocytes | | Small lymphs | Large lymphs | Monocytes |
| | | | Banded | Segmented | | | |
| Control | 34 | *per cmm.* 10,000 (7,000–15,000) | *per cent* 2 (0–5) | *per cent* 23 (10–31) | *per cent* 68 (52–80) | *per cent* 5 (2–12) | *per cent* 2 (0–6) |
| 24 hours | 8 | 3,400 (1,150–7,100) | 0 | 74 (68–84) | 25 (14–33) | 0 | 1 (1–2) |
| 48 hours | 14 | 2,300 (50–5,800) | 1 (0–9) | 88 (70–97) | 9 (0–23) | 2 (0–8) | 0 |
| 72 hours | 10 | 600 (250–1,150) | Too few cells for differential counts, but 90 per cent of the cells seen were lymphocytes | | | | |

Subcutaneous doses of *1.5 to 2.5 mg. per kg.*, permitting many recoveries, produced a temporary fall in the leukocyte count which returned to normal in 4 to 6 days. Polychromasia was decreased on the fourth to sixth day, followed by a definite increase above normal.

*Mice gassed at 1 to 3 $LC_{50}$* and dying 3 to 6 days later also showed a severe leukopenia as reported by Lushbaugh at the University of Chicago.[20, 26]

*Rats*

Blood for leukocyte counts and differentials was obtained by cardiac puncture from unanesthetized rats and at sacrifice from the free-flowing aortic blood of anesthetized animals. Control counts and differentials

(Table I) were in agreement with the data of others.[27,28] Rats were given 3 mg. per kg. of the hydrochloride intravenously (2.5 $LD_{50}$) in one series and 3.0 mg. per kg. subcutaneously (1.6 $LD_{50}$) in another series. Animals in both groups died in the period of 72 to 110 hours after administration. The rapid fall in leukocytes and the change in the differential count were almost identical in the two groups and the data obtained on the intravenous group are presented in Table I.

Rats near death at 3 to 6 days after gassing *at 1 to 4 $LC_{50}$* showed a severe leukopenia, as also noted by Lushbaugh.[26]

### *Rabbits*

Our most extensive study of the circulating blood was made on rabbits. The material is presented in some detail although it represents an incomplete study. It is organized, however, for the purpose of orientation in the problem and toward the planning of further hematologic studies.

*Normal Leukocyte and Differential Counts in the Rabbit.* Blood from the ear vein or occasionally blood taken by cardiac puncture was used. Conditions tending to produce fluctuations in the leukocyte and differential counts could not be eliminated from our study. It is known that there is a moderate variation in leukocyte count during the day; that heart blood and venous blood obtained from the ear differ in leukocyte and differential count unless the venous blood is flowing freely; and that rabbits placed on their back (*i.e.*, for cardiac puncture) show a progressive fall in leukocyte count because of a decrease in lymphocytes.[29] However, our control counts, established on 21 rabbits under a variety of conditions, were in agreement with the normal counts found by previous investigators[30–32] and the changes produced during intoxication were far out of the range of variations attributable to our unstandardized methods of obtaining blood.

Twenty-one control leukocyte counts averaged 8,400 (3,650 to 17,500) per cmm. Differential counts were made on the basis of the following classifications:

*Granulocytes*

a. Pseudo-eosinophils (corresponding to neutrophils in man)
   1. Banded cells (containing a single band-shaped nucleus and cytoplasmic granules staining with eosin)
   2. Segmented cells (containing a multilobulated nucleus and granules staining with eosin)
b. Basophils (containing many coarse granules taking the basic stain)
c. Eosinophils (easily distinguished by eosinophilic granules many times larger than the granules of pseudo-eosinophils)

*Agranulocytes*

a. Lymphocytes
b. Monocytes. (Although monocytes cannot be distinguished readily from larger lymphocytes except by the use of supravital stains, a large and indented or folded nucleus which may assume many tortuous positions is regarded as distinctive of a monocyte. While reasonably sure of this criterion, since vital stains were not used, the possibility of some error must be admitted.)

Differential counts in the rabbit show considerable variation, evident in our data as well as in those of other investigators.

*Supralethal Intravenous Doses.* Five mg. per kg. intravenously was fatal to rabbits in 36 to 94 hours and produced consistent effects on the formed elements of the blood. At *24 hours* the leukocyte count was little affected, but most of the cells were mature granulocytes, while the lymphocytes were almost absent. At *48 hours* leukopenia was severe, with 150 to 4,200 cells per cmm. Animals surviving 72 hours or longer had almost complete leukopenia, the occasional cells being mature granulocytes. An occasional normoblast or immature

TABLE II

*Effects of 0.5 mg. per kg. of $HN_2$ Hydrochloride Given Intravenously in Rabbits*

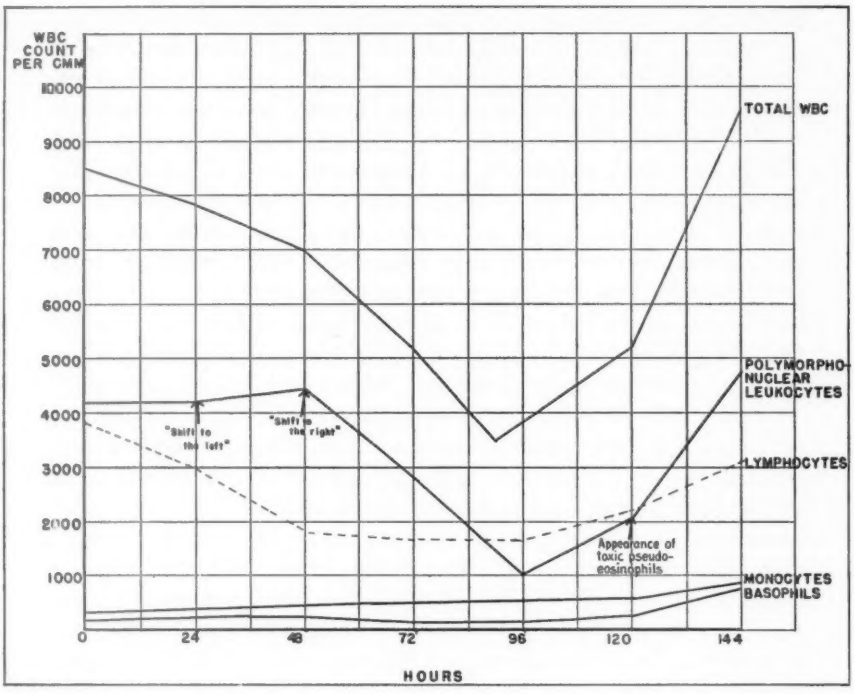
| | Total and range of white blood cell count | Differential count | | | | |
|---|---|---|---|---|---|---|
| | | Banded | Segmented | Basophils | Lymphocytes | Monocytes |
| | cmm. | | | | | |
| **Leukopenic period: 48–72 hours after injection (3 counts)** | | | | | | |
| Average and range of per cent of cells | | 3 (0–6) | 30 (10–42) | 9 (2–16) | 51 (50–52) | 7 (6–10) |
| Total number and per cent of cells | 2,300 (1,550–3,000) | 50 (0–100) | 780 (300–1260) | 110 (60–150) | 1200 (890–1500) | 160 (150–170) |
| **Recovery period: 96–120 hours after injection (4 counts)** | | | | | | |
| Average and range of per cent of cells | | 16 (8–32) | 30 (15–45) | 13 (4–39) | 31 (27–59) | 10 (8–14) |
| Total number and per cent of cells | 6,350 (4,300–7,800) | 1,050 (345–1,840) | 2,100 (680–3,500) | 800 (230–2,400) | 1850 (750–2,650) | 500 (175–870) |

granulocyte could be found within 24 hours after injection, but no abnormal forms were seen thereafter. There was a slight progressive fall in hematocrit values.

*$LD_{50}$ Doses.* Three mg. per kg. was invariably fatal and 2 mg. per kg. permitted an occasional recovery. Up to 3 to 4 days after injection the course of the leukocyte and differential counts was similar to that seen after 5 mg. per kg., with counts falling to a level ranging from 50 to 660 cells per cmm. In rabbits surviving this period, however, some recovery of the leukocytes was evident. Two rabbits receiving 3 mg. per kg. and surviving longer than 100 hours had "toxic" pseudo-eosinophils,* anisocytosis, macrocytosis, and polychromasia. (One rabbit survived 170 hours and the leukocyte count was 3,000 at the time of death.)

* We refer to polymorphonuclear pseudo-eosinophils which contain large basophilic granulations of irregular size and number, or cells that are swollen and exhibit bizarre nuclei or multisegmented nuclei, or agranular cells with basophilic cytoplasm, as "toxic" pseudo-eosinophils. Pseudo-eosinophils with basophilic granulations were the most frequent and constant form of abnormal cells present in our smears.

At 2 mg. per kg., several rabbits survived beyond 100 hours and in these evidence of recovery of the leukocyte count was more striking. Whereas at 72 to 96 hours the average of 5 counts was 325 cells per cmm., at 120 hours the average of 4 counts had risen to 1,775 (1,170 to 2,400). The differential count showed an increase in basophils,




Text-Figure 2. Effects of 1 mg. per kg. of $HN_2$ hydrochloride on the leukocyte and differential counts of rabbits.

monocytes, immature granulocytes (which were occasionally vacuolated), and many "toxic" pseudo-eosinophils. Occasional mitotic figures were found in the circulating blood. There were varying numbers of poikilocytes, anisocytes, stippled cells, and normoblasts, and polychromasia was increased.

*Sublethal doses.* A dose of 0.5 to 1.0 mg. per kg. caused a transient leukopenia followed by a rapid recovery of the leukocyte count. The effects of 1 mg. per kg. were fairly consistent and are shown graphically in Text-Figure 2 and Table II. Although leukopenia was not as severe as after larger doses, there was failure of young regenerating granulocytes to appear in the circulating blood for 72 or more hours

after injection. The lymphocyte count decreased early but not markedly when compared to the later fall in granulocytes. The rapidity of the recovery of the leukocyte count was striking at these doses, in some cases rising from 2,000 to 15,000 overnight. The nature of the cells contributing to recovery are shown in Figure 1, cells of abnormal type occurring in diminishing numbers up to 12 or 14 days after injection. There was no morphologic change in the red cells.

Gilman, Goodman, Phillips, and Allen [33] have described similar hematologic effects after intravenous, subcutaneous, or cutaneous administration of $HN_2$, and noted that recovery from the leukopenia may be followed by a secondary fall in leukocyte count. They were able to maintain a leukopenic state over a period of 18 days by daily intravenous injections of 0.5 mg. per kg. of the hydrochloride, and a slow but progressive fall in the red cell count also occurred. MacLeod and Rhoads' data [34] are also in agreement, and they particularly commented on the great numbers of pseudo-eosinophilic granulocytes containing basophilic granulations which appear during the stage of recovery from leukopenia. Kethley *et al.*[35] also induced severe leukopenia by the cutaneous application of the free base at $LD_{50}$ doses.

*Gassing.* Our hematologic data on gassed rabbits are limited, but demonstrate several important features. One rabbit exposed to 1.08 mg. per liter for 10 minutes died after 54 hours. At 24 hours the leukocyte count was 16,750; the differential count disclosed 7 per cent banded and 81 per cent segmented pseudo-eosinophils, 2 per cent monocytes, and 10 per cent small lymphocytes. At 48 hours the leukocyte count was 2,300 with only 11 per cent segmented pseudo-eosinophils, 2 per cent basophils, 80 per cent small lymphocytes, and 7 per cent monocytes. These changes in the leukocyte and differential counts parallel those seen after the parenteral administration of lethal doses of the $HN_2$, and this rabbit apparently died with a considerable degree of systemic intoxication.

Of 3 rabbits gassed at 0.5 mg. per liter for 10 minutes, one died at 5 days, the second at 9 days, and the third recovered. Both dying animals had leukopenia of about 3,000 cells per cmm. with no increase in immature cells at 48 hours. At 96 to 120 hours the leukocytes were increasing rapidly in both animals, and large numbers of immature forms were evident in the smears. One rabbit, at 120 hours, had a count of 18,940 per cmm. with a differential count of 15 per cent myelocytes, 67 per cent banded "toxic" pseudo-eosinophils, 12 per cent monocytes, and 6 per cent small lymphocytes. At autopsy it appeared that these animals had died of pulmonary infection. The surviv-

ing rabbit did not develop true leukopenia since the count ranged from 19,700 at 24 hours after gassing to 9,500 and 11,050 at 48 to 72 hours, followed by recovery to 28,000 per cmm. at 96 hours. At 48 hours granulocytes fell, but at 72 to 120 hours the count again rose with large numbers of immature forms present. This animal also developed a severe respiratory infection but recovered. Apparently gassing suppressed but did not wholly inhibit granulocyte production, and cells of regenerative type appeared promptly in the circulating blood. These variable effects may reflect variations in the amount absorbed via this route of administration because of unpredictable variations in rate and volume of respiration.

Kethley *et al.*[35] found that gassing rabbits with approximately $LC_{50}$ of $HN_2$ (0.7 to 0.9 mg. per liter for 10 minutes) produced marked leukopenia, 61 per cent of their rabbits dying within 3 days. The animals surviving longer than 3 days showed recovery of the leukocyte count, but half of them died 5 to 6 days after gassing. The cause of death was not discussed by the authors. Lushbaugh [26] reported leukopenia in gassed rabbits that subsequently died with pulmonary abcesses and pneumonia after the hemopoietic tissue had recovered and leukocytosis was present.

*Other Formed Elements of the Blood.* Rabbits dying of $HN_2$ intoxication do not consistently become anemic, although there has been a slight fall in hematocrit value in most of our animals which was no doubt accentuated by occasional bleeding. Kethley *et al.*[35] found no significant changes in the red blood cell count, hemoglobin, and platelet count of gassed rabbits, but they reported that the normal reticulocyte count (4 per cent) fell sharply within 2 days, and we have noted a disappearance of polychromasia. These two facts indicate that at lethal doses erythrocyte production is arrested. The circulating red cells appear unaltered and presumably are capable of living out their life span of 40 to 140 days; since in surviving animals recovery of the hemopoietic tissue occurs within less than 1 week, the hiatus in red cell formation does not generally cause a significant decrease in red cell count or hematocrit.

At $LD_{50}$ dosage reticulocyte formation is depressed up to 4 days, to be followed by the appearance of normoblasts, increased polychromasia, macrocytes, anisocytosis, and poikilocytosis.

It appears from the available data that adult cells in the blood, possibly excepting lymphocytes, are unaffected, the essential fault in intoxicated animals being a failure of cellular replacement in the blood-forming organs, so that those cells having the shorter life span dis-

appear first. The sequence, therefore, is an early fall in lymphocytes and a slow fall in granulocytes and reticulocytes, but only a very slight decrease in red cells and hemoglobin.

The abnormal leukocytes in the peripheral blood are somewhat similar to those seen following the extreme bone marrow stimulation induced by withdrawing leukocytes from the circulating blood by means of the peritoneal exudate technic.[36] The occurrence of polycytes and macropolycytes is apparently due to an increase in the rate of maturation of the polymorphonuclear leukocytes, and their presence has been described after various drugs and extracts, x-rays, ultraviolet light, thyroid extract, peritoneal exudates, and infection.[37]

Following intoxication of the marrow at $LD_{50}$ dosage, superimposed infection appears to be ineffective as a bone marrow stimulant in preventing the leukopenia from inducing early regenerative activity of the bone marrow; we have observed an early shift to the left in only one of 3 rabbits gassed at a sublethal concentration. Dr. J. MacLeod (personal communication) has been unable to induce leukocyte-containing peritoneal exudates 24 hours after the intravenous injection of $HN_2$ HCl, and similarly the leukocytosis-producing factor of Menkin given after the intoxication does not prevent the leukopenia or protect the bone marrow of dogs.[34] The injection of the leukocytosis-producing factor prior to intoxication in one dog appeared to prevent bone marrow aplasia and leukopenia.[34] On this basis, the presence of a pyogenic infection at the time of injection may be expected to modify the effect of $HN_2$ on the bone marrow. A protective effect of infection on the bone marrow injury induced by benzene has been reported.[38]

Cameron and Short [21] demonstrated an increase in sedimentation rate, a decrease in coagulation time, and no change in the red cell fragility in rabbits intoxicated by subcutaneous injection of 3 mg. per kg. of the free base.

*Other Species*

MacLeod and Rhoads [34] observed that *dogs* receiving 2 mg. per kg. of the hydrochloride intravenously developed lymphopenia while the neutrophil count did not fall markedly. At autopsy, however, the bone marrow was aplastic, suggesting that circulating neutrophils are longer-lived in the dog than in the animals reported above. Cameron and Foss [17] observed leukopenia in *goats* following cutaneous application of the free base, and Irwin, Brackenbury, and Young [39] have made similar observations in the *monkey*. The demonstration of leukopenia in gassed guinea-pigs, cats, and dogs [26] has failed frequently, perhaps because of early death from respiratory injury. In dogs under nembutal anesthesia with the thoracic duct cannulated, the lymph output during

the first 5 hours was at first increased and then decreased, while the lymphocyte content of the lymph decreased. This circumstance resulted in a normal lymphocyte output during this period. One, 2, and 3 days after intoxication, lymph flow was only one-half of normal and the cell count was very much less than normal. Since the decrease in circulating lymphocytes was greater than the decrease in output of lymphocytes, it was concluded that there was an increased disappearance of lymphocytes from the blood in some unexplained fashion.[40] However, this conclusion should be weighed against the known short life of circulating lymphocytes.[41]

TABLE III

*Leukocyte and Differential Counts Following 3.0 mg. per kg. of HN2 Hydrochloride Given Intravenously to a Hen*

| Days after injection | Total leukocyte count | Differential counts | | | | |
|---|---|---|---|---|---|---|
| | | Granulocytes | | | Agranulocytes | |
| | | Segmented | Basophils | Eosinophils | Lymphocytes | Monocytes |
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Control | 21,090 | 71 | 4 | 2 | 23 | 0 |
| 1 | 49,600 | 85 | 1 | 2 | 6 | 6 |
| 2 | 8,200 | 65 | 4 | 8 | 16 | 7 |
| 3 | 6,000 | 50 | 6 | 7 | 33 | 4 |
| 4 | 2,000 | 88 | 7 | 3 | 0 | 2 |
| 5 | 20,550 | 44 | 4 | 3 | 46 | 3 |
| 6 | 24,800 | 41 | 5 | 3 | 48 | 3 |
| 8 | 25,000 | 49 | 3 | 5 | 40 | 3 |

In the *dog* there is a suggestion that the fall in the granulocytes is slower. Animals given 2 mg. per kg. intravenously died at a time when the granulocyte count was relatively high, although there was almost complete depletion of myeloid tissue at death. This circumstance was attributed to the longer life cycle of the canine granulocyte.[34] This delayed granulocytopenia seems confirmed in dogs receiving 1 mg. per kg. intravenously,[4] but in dogs given 1, 2, and 3 mg. per kg. subcutaneously it is stated that granulocytopenia appears *pari passu* with bone marrow injury and that the granulocyte counts reached low levels in 3 days.[40]

Leukopenia has been induced in *chickens* by sublethal doses of the hydrochloride ranging from 3 to 10 mg. per kg. intravenously. The leukocyte and differential counts on 2 hens receiving 3.0 and 7.5 mg. per kg. are tabulated in Tables III and IV. That these effects were not due to the moderate weight loss suffered by these animals was shown by a control bird that was starved for 6 days without the development of leukopenia. Similar leukotoxic action was observed after injection of H into chickens.

In *mice* surviving the culmination of hemopoietic injury, regeneration is delayed for several more days. Meanwhile, hemopoietic centers in other organs, notably the liver, show marked evidence of stimulation, so that even in animals dying between the fourth and seventh days some restoration of the leukocyte count may occur prior to death. In surviv-

TABLE IV

*Leukocyte and Differential Counts Following 7.5 mg. per kg. of HN2 Hydrochloride Given Intravenously to a Hen*

| Days after injection | Total leukocyte count | Differential counts | | | | | |
|---|---|---|---|---|---|---|---|
| | | Granulocytes | | | | Agranulocytes | |
| | | Banded | Segmented | Baso-phils | Eosin-ophils | Lymphocytes | Monocytes |
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Control | 32,650 | | 39 | 9 | 2 | 49 | 1 |
| 1 | 23,600 | | 96 | 2 | 0 | 1 | 1 |
| 2 | 1,550* | | 44 | 9 | 13 | 34 | 0 |
| 3 | 2,000* | 25† | 6 | 19 | 0 | 44 | 6 |
| 4 | 5,100* | 20† | 0 | 5 | 5 | 35 | 35 |
| 5 | 8,300 | 13† | 0 | 18 | 5 | 55 | 9 |
| 6 | 14,500 | 2 | 12 | 6 | 7 | 68 | 5 |
| 7 | 15,850 | 7 | 19 | 4 | 4 | 65 | 1 |
| 8 | 11,000 | | 10 | 4 | 4 | 47 | 34 |
| 11 | 42,000 | | 43 | 2 | 1 | 53 | 1 |
| 19 | 21,300 | | 52 | 11 | 10 | 21 | 6 |

* Too few cells in the smear to permit a satisfactory differential count, and the figures presented are only approximate.

† Immature cells of the myeloid series not ordinarily seen in the circulating blood.

ing animals the lymphocytes appear to recover before the granulocytes. Histologically, marked hyperplasia in the thymus and lymph nodes parallels this recovery. Granulocytic recovery is spectacular in the blood, the white blood cell count rising from leukopenic to normal, or even supra-normal levels, almost overnight. This phenomenon appears to be related to the outpouring of immature granulocytes, and regenerative activity may persist for 2 to 3 weeks. In mice, a species particularly sensitive to overstimulation of the leukopoietic tissue, hyperplastic foci having the dimensions and intensity of a leukemoid reaction have been observed after an interval of from 3 to 4 weeks.

### BLOOD STUDIES AFTER H

A reduction in the number of white cells as well as various biochemical changes in the blood follow parenteral injection or cutaneous application of $LD_{50}$ or greater doses of H. Blood platelets are markedly decreased only in severely intoxicated animals.[42] In the early phase the red blood cells apparently are little affected. The reduction in white blood cells involves both granulocytes and lymphocytes; the granulo-

cytes usually are somewhat more sensitive. Leukopenia may be preceded by a nonspecific(?) *leukocytosis* lasting a day or more.[33] In animals *gassed* with H, leukocytosis, leukopenia, and lymphopenia may or may not occur, probably reflecting variations in actual dosage inhaled.

Hemoconcentration indicated by increased red blood cell count, percentage of hemoglobin, and hematocrit value may rapidly follow intoxication. This has been observed in various species after parenteral, topical, and vapor intoxication.[43]

In surviving animals at a time when the leukocyte count may be returning to normal or above, a progressive anemia may occur with no alteration of the reticulo-endothelial system.[43] In rabbits and dogs this anemia frequently resembles the idiopathic or benzol type. The hematocrit reading, percentage of hemoglobin, and red blood cell counts decrease with no change in the color index, mean corpuscular volume, or diameter as measured by the Price-Jones method. Thus it appears that the marrow produces fewer red cells for a time, and that in some instances the cells are immature. The delayed anemia may reflect an initial injury of the erythroblastic tissue which, because of the longer life of the red blood cells, is not evident during the first few days of intoxication. Intestinal hemorrhage also may contribute to the anemia.

## HISTOPATHOLOGIC SYSTEMIC EFFECTS

### *Bone Marrow*

One of the most specific features of $HN_2$ or H intoxication thus far encountered is the depletion of the bone marrow, also described by Zimmerman,[9] Cameron and Short,[21] Lushbaugh,[26,43] and Warren, MacLeod, and Rhoads.[44] Sternal and femoral marrow have been examined regularly by us, except in a few instances in which we were limited to the cranial or vertebral marrow, but we have seen no differences in marrow chosen from different sites. Obvious bone marrow depletion does not occur for 24 to 48 hours, irrespective of the dosage, but rats and rabbits receiving lethal doses showed degenerative cellular changes when studied 8 to 30 hours after injection. These changes consist of swelling and dissolution of hemopoietic cells, alterations in their staining reactions, and the presence of enlarged vesicular nuclei and some scattered débris. Mitotic figures can be seen up to 8 hours and are usually absent thereafter until recovery. Agglomerated masses of eosinophilic granules without nuclei may be observed early. In other cells the nuclei are also absent, but scattered throughout the cells are numerous dark-staining chromatin particles suggesting disruption of the nuclei. Suggestive alterations are seen in the megakaryocytes,

consisting of bizarre swellings, but the reticular elements remain unchanged. Warren and Rhoads [45] have found the injured cells to be very friable, and easily damaged when smeared.

Following this period and varying to some extent with dosage and possibly with the route of administration, depletion of the marrow progresses rapidly to almost complete aplasia in 40 to 90 hours (Figs. 2 and 3). All hematopoietic cells disappear and there is neither shift to the right or left, nor loss or accumulation of specific types. The cells remaining in the marrow are those lining the blood vessels, some reticular cells, fibrocytes, rare macrophages, fat cells, and possibly primitive erythrogenic elements (hemocytoblasts [26]). There is a tendency for endosteal stem cells to persist, as Dr. C. E. Dunlap (personal communication) has observed in mice injured by x-rays. The megakaryocytes are conspicuous for their bizarre, pyknotic nuclei, and for their persistence in a marrow depleted of its hemopoietic tissue. Chromatin débris is sometimes seen in earlier periods, but it is usually scanty or absent. The marrow is replaced by a loose spongy mass of acidophilic tissue with protein precipitate, fat cells, and dilated sinusoids engorged with erythrocytes.

With large doses animals may die before marrow depletion is complete, but in delayed deaths, as seen in mice, rats, and rabbits at $LD_{50}$, the bone marrow is invariably hypoplastic and usually aplastic at death. This appears to be true also in dogs.[45] Irwin, Brackenbury, and Young,[89] however, have described only slight degenerative changes in the marrow of monkeys receiving fatal dermal applications of the free base, although the animals became leukopenic. Typical depletion of the marrow follows gassing of mice and rats at $LC_{50}$.

Animals escaping delayed deaths begin to show regenerative activity throughout the depleted marrow in 4 to 5 days after injection. There is a marked shift to the left as the new stem cells appear,[45] and mitotic activity becomes evident. During recovery the marrow of rabbits fails to show the normal pseudo-eosinophilia of the granulocytes and Warren and Rhoads [45] have described abnormal vacuolated cells. These changes may be correlated with the immature cells and "toxic pseudo-eosinophils" described in the circulating blood during the recovery phase. Death may occur when the bone marrow is partially or completely regenerated, but it is our impression that these remote deaths are not due to direct action of $HN_2$.

### *Lymphoid Tissue*

Involution of the lymphoid tissue is one of the earliest and most striking pathologic effects of both the N and S mustards. The rapidity of this involution was quantitatively determined in rats receiving 3 mg.

per kg. (1.6 $LD_{50}$) of $HN_2$ HCl subcutaneously and sacrificed by exsanguination 24, 48, and 72 hours after injection. The weights of the spleens, thymuses, and lymph nodes are shown in Table V. Microscopic observations on these organs were made chiefly in mice and rats, with less complete but analogous observations in rabbits.

TABLE V

*Average and Range in Weights of Lymphatic Organs in Rats Intoxicated with $HN_2$*

| Hours after injection | No. rats | Average body weight at death | Spleen | Thymus | Cervical lymph nodes |
|---|---|---|---|---|---|
| | | *gm.* | *mg.* | *mg.* | *mg.* |
| Controls | 8 | 178 (169–192) | 865 (600–1260) | 155 (110–206) | 73 (45–100) |
| 24 hours | 5 | 172 (164–184) | 541 (375–804) | 102 (73–139) | 55 (53–56) |
| 48 hours | 4 | 163 (150–178) | 446 (337–536) | 79 (61–100) | 49 (34–70) |
| 72 hours | 8 | 162 (150–185) | 233 (168–285) | 39 (33–61) | 23 (15–30) |

### *Lymph Nodes*

The lymph nodes, as in the case of the thymus, show a variable degree of lymphocytic fragmentation, large doses producing intense fragmentation visible in 10 to 48 hours, while small doses cause milder effects. All animals, even at $LD_{50}$, show loss of follicles and general contraction of the lymph nodes, and on microscopic examination these nodes are seen to be composed of epithelioid cells * and condensed reticular and fibrous elements, with almost complete absence of lymphocytes. As in the case of the spleen and thymus, there is no interference with the mobilization of macrophages and epithelioid cells. Lymph nodes examined up to 3 weeks after intoxication do not show any evidence of hemopoiesis, although lymphocytic hyperplasia was present.

### *Spleen*

This organ likewise exhibits changes which are roughly proportional to dosage. Dosage in excess of $LD_{50}$ given by any route leads to prompt fragmentation of lymphocytes in the malpighian corpuscles and to a lesser extent in the interstitial tissue of the so-called red pulp (this is best seen in mice), similar to that seen in the lymph nodes and thymus. With time, much chromatin material is found within macrophages in all parts of the spleen. The progressive reduction in the size of the spleen (Fig. 4) results from the disappearance of lymphocytes with atrophy of the corpuscles and loss of cells from the interstitial tissue of the sinus walls. In mice, after smaller doses, megakaryocytes, which are notable in the normal spleen, may increase in number. These often show bizarre, enlarged, pyknotic nuclei, and monolobulated forms are more

* Designated as epithelioid cells because they are large, contain abundant eosinophilic cytoplasm, and appear pavement-like; these cells closely resemble macrophages and may be the same cells, differing only in the absence of readily discernible phagocytized material.

common than multilobulated cells. At 48 hours the disappearance of lymphocytes from the corpuscles and from the red pulp gives the organ a somewhat fibrous appearance. At the periphery of the malpighian corpuscles epithelioid cells or fibroblasts are seen, and occasionally small foci composed of similar cells may be found apart from the corpuscles.

During the first 2 days of intoxication, intact polymorphonuclear leukocytes or banded forms, and occasionally eosinophilic myelocytes, may be seen in a spleen in which the lymphocytes have undergone intense fragmentation or sharp reduction in number. The ultimate stage in the regression of the spleen is reached at 70 to 100 hours, at which time the neutrophils also have disappeared. Necrotic changes in the corpuscles may no longer be visible, although nuclear débris may still be found in occasional macrophages. In some animals a small number of lymphocytes may persist, but the malpighian corpuscles are composed largely of reticular cells.

At $LD_{50}$ doses or less, the spleen may appear fairly normal in the first 48 hours, except for a variable increase in megakaryocytes, but thereafter it undergoes progressive contraction. Polymorphonuclear leukocytes are progressively reduced in number from about 40 hours onward until recovery begins. How this is accomplished is not clear, for no increase in chromatin débris is visible. After 5 or 6 days the animals that have survived at $LD_{50}$ frequently show excessive myelopoiesis; the corpuscles thereafter enlarge, and contain a rich mixture of lymphocytes with reticular cells, although the former are overwhelmingly preponderant. In the red pulp, clusters or cords of primitive hemopoietic cells reappear and polymorphonuclear leukocytes are found in large numbers around these areas. Plasma cells are usually conspicuous in these foci. There is little evidence of hemolysis, as indicated by storage of hemosiderin in the macrophages, and when present there is a question whether this pigment represents coincident or antecedent hemolysis.

*Thymus*

Doses considerably in excess of $LD_{50}$ regularly produce marked thymic necrosis which is best seen from 12 to 48 hours after administration. At $LD_{50}$ or less, the thymic effects are more gradual in their evolution, especially in rats and rabbits as compared to mice, but the organ contracts (Fig. 5) with more or less complete replacement of the cortex by reticular or epithelioid cells. After gassing or oral administration, in the range of $LD_{50}$, the effects on the thymus are less striking than after parenteral administration, and usually consist of moderate atrophy with little lymphocytic fragmentation.

The lymphocytic fragmentation observed after large doses generally occurs by karyorrhexis; karyolysis or fading out of the nuclear structure has been less frequently observed, and is most evident in animals that do not show much karyorrhexis. Lymphocytic fragmentation progresses to an advanced stage within 24 hours. The nuclear débris is usually free in early stages, but macrophages appear later and most of the material is finally engulfed within the macrophages or disposed of by other means.

When the thymus undergoes involution, the cortex shrinks somewhat more rapidly than the medulla, and during the period of cortical contraction the lymphocytes may appear more numerous in the medulla than in the cortex, thus tending to reverse the normal pattern. In surviving animals this reversal may be corrected, the cortex reassuming its normal lymphocytic appearance, while the medulla becomes conspicuous for its reticular cells and epithelial elements. The profoundly involuted thymus, found 72 to 96 hours after $LD_{50}$, is small, fibrous, and stringy in appearance, while in other animals surviving $LD_{50}$ or slightly less, the thymus may exhibit hyperplastic changes at 90 hours or later, and in many animals examined after 1 week the organ appears to have undergone an increase in size.

Changes similar to those observed by us in the lymphatic tissues have been recorded by Zimmerman,[9] Cameron and Short,[21] Lushbaugh,[26] and Irwin, Brackenbury, and Young.[39] The early and graded injury of lymphopoietic tissue, and its rapid recovery after $LD_{50}$, is correlated with changes in the number of circulating lymphocytes. Lymphopenia occurs precipitously during the first 24 hours and may become extreme if intoxication is fatal. At lower doses the count may show a plateau after 24 hours and begin to rise on the fourth to fifth day. There is no correlation between lymphopenia and the ensuing granulopenia, in that at low doses lymphocytes show an immediate moderate but reversible fall, while on the third to fourth days a severe granulopenia may appear. Also, lymphocytes, although affected earlier, are not necessarily more sensitive than the bone marrow cells as far as the ultimate severity or duration of effect is concerned.

### *Intestinal Tract*

Clinical disturbances and gross alterations in the intestinal tract of intoxicated mice, rats, and rabbits, and histologic preparations of rolled-up segments of the entire intestines in serially sacrificed mice, rats, and rabbits receiving 1 to 2 $LD_{50}$ doses demonstrate consistently the presence of extensive intestinal injury. The lesion starts just below the pylorus and extends the entire length of the small intestine, with the

ileum most severely involved. The stomach and Brunner's glands appear unaffected, but microscopic focal epithelial lesions may be found in the first part of the colon.

The early process is degenerative and inflammatory (Fig. 6-B), involving most of the mucosa, with epithelial injury and erosion being marked. Within 24 to 48 hours after injection the villi become edematous and the lining cells appear swollen and may be distended with clear vacuoles, leading finally to cellular rupture and desquamation. The mucosa may show edema and fibrin beneath the lamina propria, hyperemia, dilation of the lymphatics, and a variable inflammatory reaction consisting largely of polymorphonuclear leukocytes and eosinophils. Necrotizing changes in the interstitial cells, including the lymphocytes, are not conspicuous. Rats given trypan blue intravenously or intraperitoneally exhibit selective deposition in the mucosa of the small intestine after 48 hours.

At 72 to 96 hours, when the lesion is most severe, a mixture of extensive injury, metaplasia, and regenerative activity of the epithelium is seen (Figs. 6-C and 6-D). The crypts of Lieberkühn appear as cyst-like spaces lined by flat, elongated cells, and occasionally contain an accumulation of polymorphonuclear leukocytes, nuclear débris, and desquamated cells. In other areas the crypts may be lined by hypertrophic, hyperchromatic cells. The mucosal surface may be denuded, or show patchy areas of metaplasia, or epithelial hypertrophy and hyperplasia with vesicular swelling of the nuclei. The regenerating cells are flattened and occasionally show surface spines. They usually contain hyperchromatic nuclei composed of large chromatin masses. Mitotic figures are common. Goblet cells show increased mucus formation, especially in the colon. The maximal epithelial alterations and sloughing coincide in time with the development of fluid distention and mucous diarrhea. In later stages a slight fibrous reaction may lead to deposits of connective tissue in the villi which tend to become shorter, broader, and stubby. Rats show these intestinal changes more strikingly, but they are also apparent in mice and rabbits.

The significance of the functional and pathologic changes in the intestinal tract in relation to death of the animals, whether by loss of fluid and alkali, by increased permeability of the intestinal wall to injurious substances, or by the lowering of the local defense mechanism against the invasion of bacteria, raises problems treated elsewhere.[4]

### *Respiratory System*

Animals receiving 1 to 3 $LD_{50}$ parenterally do not show any consistent or severe injury to the upper or lower respiratory tract. Guinea-

pigs receiving large doses subcutaneously are reported to develop pulmonary edema, but not after intraperitoneal injection.[12] Cameron and Foss [17] have described, in goats, edema and congestion of the lungs, and gross congestion and hemorrhages in the trachea and larynx after subcutaneous injection of large doses. Pulmonary edema has been seen occasionally by us in rats receiving the hydrochloride intravenously, especially in those subjected to an abdominal operative procedure at the time of injection, but the phenomenon has not been examined in detail.

### *Circulatory System*

Animals injected parenterally do not show any consistent injury to the heart or blood vessels.

### *Excretory System*

None of our animals has shown any specific injury to the kidneys or urinary bladder. Lushbaugh [26] has found infiltration and parenchymatous degeneration, *e.g.*, nuclear fading, pyknosis, and cytoplasmic sloughing, in the proximal and distal convoluted and collecting renal tubules, especially in mice and rats, but such changes as we have seen do not exceed those observed in fasted nonintoxicated animals.

### *Reproductive System*

Some sections of mouse testes have suggested maturation arrest in spermatogenesis, but to evaluate injury to the generative organs, better controlled stock than we possessed at the time of observation is required. The picture is complicated by the severe weight loss, which is known to diminish pituitary activity.[14] We have noted that following an $LD_{50}$ of the hydrochloride, surviving female mice do not become sterile, and that chickens can resume laying eggs.

### *Endocrine System*

The pituitary gland was not studied. The thyroid and pancreas are not affected, but in the rat the adrenals are increased 60 per cent in weight and cortical hypertrophy and depletion of lipid are noted. The adrenal hypertrophy may be comparable to that described by Selye [46] in rats subjected to a variety of noxious stimuli.

### *Liver*

No impressive changes have been seen in the livers of any of the species studied. Glycogen depletion is fairly common, especially in animals receiving large doses, and slight fatty infiltration is noted 3 to 4 days after injection, especially in mice. Even after oral administra-

tion (when lethal doses are absorbed partly into the hepatic-portal system to be routed directly into the liver), or after intrasplenic injection (when the material is circulated directly through the liver) there is no obvious injury of the liver parenchyma. Zimmerman,[9] however, reported focal necroses in the livers of cats but not rats after the oral administration of the hydrochloride.

Lushbaugh [26] also has described fatty infiltration in the mouse liver 4 to 6 days after gassing. This increased fat may be attributable to progressive anorexia and fasting, since Hodge *et al.* [13] have shown in fasted mice that the total liver lipid increases to 2 to 3-fold in the first day and decreases to normal on the third to fourth days; coincidentally, however, the liver rapidly decreases in size, so that the percentage of fat in the liver is usually above normal. We have observed also an increase in histologically demonstrable fat in the livers of starved control mice.

### COMPARISON WITH EFFECTS OF H

For producing visible systemic injury in rats, H is superior at $LD_{50}$ doses to the β-chloroethyl vesicants (HN1, HN2, and HN3), when administered intravenously or subcutaneously. In a comparative study of the delayed systemic action of H, HN1, HN2, and HN3, rats were exposed to these compounds via cutaneous application, gassing, intravenous and subcutaneous injection at dosage in the range of the $LD_{50}$ or $LC_{50}$, and about 0.5 $LD_{50}$ or 0.5 $LC_{50}$. Weight loss, leukopenia, lymphoid atrophy, myeloid changes, and enteritis were measured or sought in each animal. Almost all were sacrificed at 72 or 96 hours after exposure to, or administration of, the agents; a few were included at 48 and 120 hours after exposure. Wide variations occur between the intravenous and subcutaneous routes when compared with the effects following cutaneous application or gassing. Text-Figure 3 shows the intensity of total systemic injury and of the individual lesions following the levels of dosage used. After gassing and percutaneous application, the systemic effects of HN compounds, at both levels of dosage, were more severe than after H, with the single exception that HN1 was less myelotoxic than the other three compounds. On the basis of dosage required, HN3 was the most efficient in producing delayed systemic effects. Intravenous and subcutaneous injection of H (in propylene glycol) reverses the relationship to the HN compounds; at the $LD_{50}$ dosage it was most consistent and toxic in its action, judging by the loss of weight, leukopenia, extent of lymphoid atrophy, bone marrow injury, and enteritis. HN3 was again the most efficient of the nitrogen mustards; HN1 was the least leukotoxic at the dosage employed. At the sub-$LD_{50}$ or sub-$LC_{50}$ dosage these differences among

the compounds were maintained with all routes of administration but were not so striking.

### COMPLICATIONS AND VARIATIONS IN INTOXICATED ANIMALS

Some of the unusual effects and complications observed after administration of the $HN_2$ are worthy of record. These include occurrence of reactivated or superimposed infection and its relation to remote deaths, the appearance of extramedullary myeloid metaplasia in mice during recovery, and a hemolytic episode in a single rabbit.

#### *Infection*

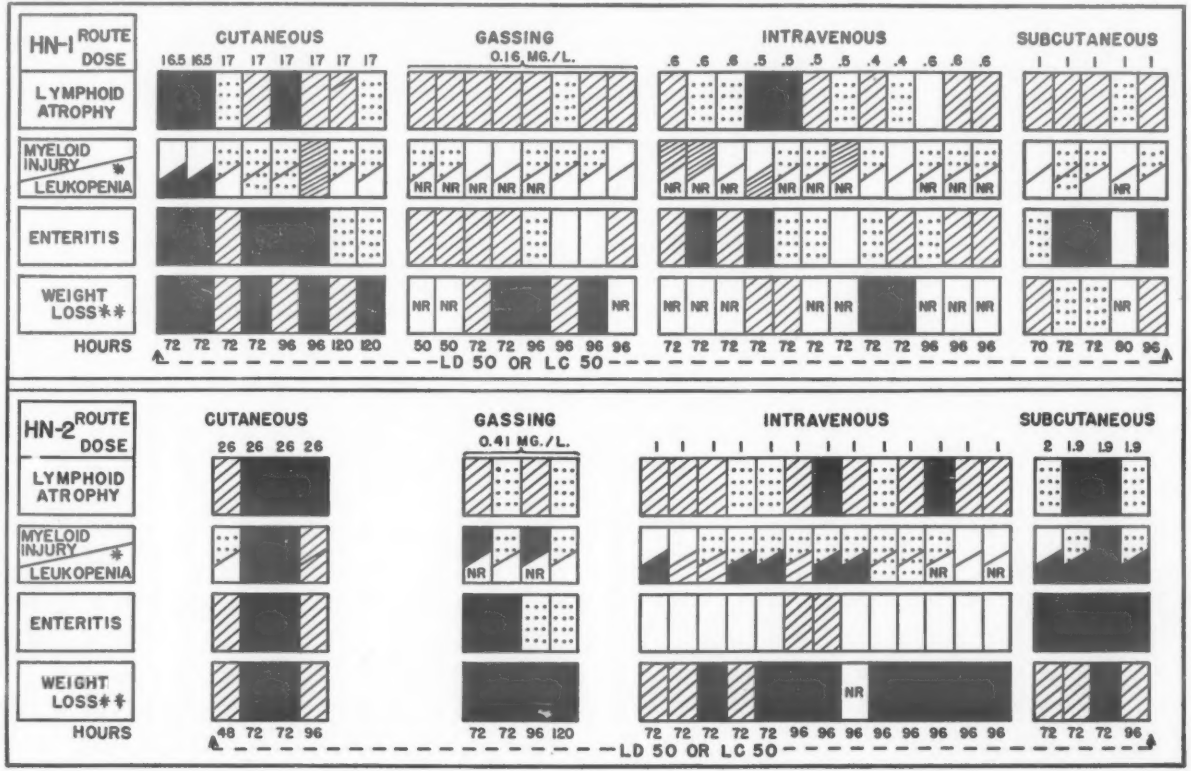
The frequent presence of obvious infection (which must heighten our suspicions as to the existence of occult infection) has been alluded to several times. In those occasional deaths occurring 7 days or longer after the parenteral injection of the hydrochloride ("remote deaths") it is necessary to rule out infection before a direct but remote toxic action can be considered. Early in our work such remote deaths were rare in mice, but about 2 months later mouse "typhoid" appeared in our colony, following which the number of remote deaths in intoxicated animals increased sharply. In many instances multiple necroses of the liver were present in these animals. Unfortunately, bacteriologic examination could not be made at the time.

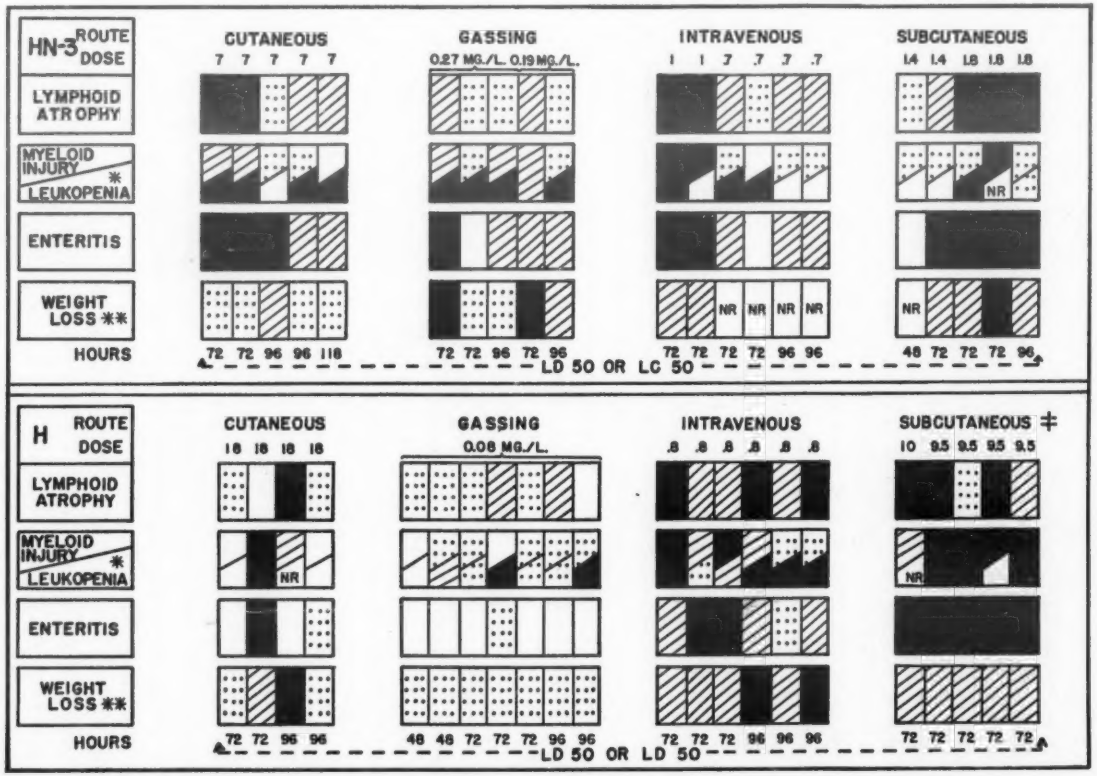
Complete pathologic studies were available in a few rabbits dying 4 days or longer after intravenous injection, at which time their leukocyte counts showed recovery. The pathologic diagnoses included pneumonia with septicemia, infected thrombi and abscesses in the lungs, heart, liver, kidneys, and gonads, necrotizing bronchopneumonia, and reactivation of coccidiosis in the intestine. The last is a pathologic rarity, but is described also after exposure of rabbits to x-rays [47] and mustard(H).[7]

It is not surprising to find secondary infection much more commonly following gassing, since the combination of severe local respiratory injury, systemic intoxication, and leukopenia present circumstances favorable to the development and spread of infection. Such infections are usually in the respiratory tract, and are particularly common in rabbits as compared to mice and rats.

All remote deaths, however, need not be attributed to infection alone. The marked weight loss, well within the range of fatal weight loss due to simple starvation, suggests that starvation may be an important and perhaps decisive factor in some cases. It is not clear whether starvation is due to debility produced by the primary intoxication which prevents the animal from eating, to changes in the endocrine and nerv-

Text-Figure 3. Comparative effects of nitrogen and sulfur mustards via various routes of administration when given at $LD_{50}$ or $LC_{50}$ dosage in albino rats. The dosage, except in the gassing experiments, is in mg. per kg. (Unshaded = no injury; dotted areas = mild injury; diagonal ruling = moderate injury; solid black = severe injury.)






* 4,000 to 2,000 white blood cells per cmm. graded as mild; 2,000 to 1,000 white blood cells per cmm. graded as moderate; 1,000 or less white blood cells per cmm. graded as severe.

** Weight loss of 2 to 9 per cent regarded as mild; 10 to 20 per cent regarded as moderate; 20 to 30 per cent regarded as severe.

‡ H in propylene glycol.

ous systems, or digestive tract, or to undetected infection which adds to and complicates other debilitating factors.

*Extramedullary Myeloid Metaplasia*

Mice surviving $LD_{50}$ doses show active hemopoiesis in the liver and spleen, this process sometimes assuming the intensity of a leukemoid reaction, and being markedly developed at 1 month. We have not followed our surviving mice beyond 1 month in systematic pathologic studies, but some mice surviving an $LD_{50}$ dose administered subcutaneously were sacrificed after 1 to 5 months and showed variable degrees of extramedullary myelopoiesis. In some animals regression had occurred. Rabbits surviving longer than 7 days after the injection of the hydrochloride have shown evidence of active hemopoiesis in the spleen.

Similar extramedullary hemopoiesis, presumably of a compensatory nature, has been described in mice and rabbits after a wide variety of procedures, including repeated bleeding, the injection of various poisons such as saponin, pyrogallol, phenylhydrazine, and benzol, and the injection of live or dead bacteria.[48] It is unlikely, therefore, that the appearance of myeloid metaplasia after $HN_2$ represents a specific effect, or that it necessarily presages the possible later development of leukemia.

*Hemolytic Episode*

Of a large group, a single rabbit receiving 2 mg. per kg. of $HN_2$ hydrochloride developed hemolytic anemia, as evidenced by a marked fall in the hematocrit value and red cell counts and an icteric plasma. Death occurred with bronchopneumonia 9 days after injection. This recalls isolated hemolytic episodes associated with various drugs, *e.g.*, sulfonamide compounds.

SPECIES DIFFERENCES

While the data are not numerous, all mammals studied appear to possess about the same general degree of susceptibility to the parenteral administration of $HN_2$. The gassing $LC_{50}$ for different species spreads over a wider range, but this may be accounted for in part by the greater susceptibility of the respiratory tract in large animals, variations in respiratory pattern, and differences in local fixation and absorption. The rat appears to be the least resistant. It is only 70 per cent as resistant as the mouse by any route of administration including gassing. Newborn rats possess no special resistance to subcutaneous injections of $HN_2$.

Chickens and pigeons possess considerable resistance to the in-

travenous injection of the hydrochloride. The $LC_{50}$ in chickens is about 10 mg. per kg. At this dose chickens may show some weakness and moderate weight loss which is slowly recovered, or more severe weakness with incoordination which usually ends fatally within a few hours. The picture of delayed death which has been described in mammals is not found in the chicken. Pigeons also may survive 10 mg. per kg. intravenously, but neurologic symptoms are more marked, weight loss is considerable, and the birds may die several weeks after injection. The $LD_{50}$ is less than 10 mg. per kg. in pigeons. The resistance of chickens is the more notable since severe leukopenia occurs after doses of 3 to 10 mg. per kg. intravenously. At none of the tested doses did diarrhea or enteritis appear. A similar degree of resistance was not found in a single experiment using H in chickens, but the cause of death was not determined in this experiment nor were post-mortem studies included.

## A COMPARISON OF THE EFFECTS OF BENZENE AND RADIATION WITH THOSE OF HN2

Since HN2 appears to be highly injurious to the bone marrow, it is of interest to compare its effects with those of two well known leukopenic agents, benzene and radiation (x-ray, radium, and neutrons).

### *Benzene*

A single lethal dose will produce an early death, with neurologic or hemorrhagic manifestations, but without leukopenia, while a single sub-lethal dose may cause a transient stimulation of the bone marrow. However, repeated doses of 1 to 2 cc. per kg. daily subcutaneously produce a progressive leukopenia which may approach an almost complete absence of leukocytes in 4 to 10 days.[48-50] Simultaneously, the lymphoid tissue shows fragmentation and involution, followed by aplasia of the bone marrow. The marrow injury consists of visible pyknosis and fragmentation of all hemopoietic cells. If the injections are continued after severe leukopenia is present, the animals will die. The cause of death is not clear, although it is known that infection plays an important rôle. If benzene is stopped when the leukopenia is severe, recovery may be rapid, and ordinarily there are no obvious sequelae. Aside from an inconstant weight loss, no notable symptoms are present, even during the leukopenia. At autopsy, the atrophic lymphatic tissue and marrow, and occasional degenerative changes in the kidneys and liver are the only evident lesions, the intestinal tract being normal. The leukopenia prevents the phagocytosis of invading bacteria [51] and, since antibody formation is also diminished,[52] benzene-

treated animals are markedly susceptible to infection.[53] With the exception of the presence of leukopenia, lymphoid and bone marrow injury, benzene poisoning is thus seen to differ in many respects from $HN_2$ intoxication.

*Radiation*

The destructive action and the extensive and prolonged alterations induced in tissues by the direct application of x-rays are well-known, and recently have been the subject of a series of reviews by Warren and his colleagues.[54] Animals exposed to sufficiently large doses of "total body" radiations survive 4 to 7 days, and then succumb to "general intoxication."[55-58] Although variation exists among species, the following generalizations may be made: There is a short latent period following which anorexia, progressive weight loss, diarrhea, and extreme prostration lead to death. Lymphocytes and then granulocytes disappear from the circulating blood within 3 to 4 days, but anemia develops slowly. No biochemical changes in the blood incompatible with life have been found,[54] although generally there is evidence of an increase in protein catabolism.[56] At autopsy the lymphatic organs are atrophic, the intestines may be ulcerated, hemorrhagic, and distended, and the bone marrow is aplastic. Histologically, the lymphatic tissue appears to be most sensitive, and early necrosis and fragmentation of the lymphocytes and involution of the spleen and thymus are found. The marrow shows early damage by loss of staining properties and lysis, or by pyknosis and fragmentation of nuclei, but aplasia is not complete for 2 to 3 days. Extensive ulcerations and epithelial changes occur in the intestine. These effects are due to the direct action of the x-rays,[54,59] although trauma and secondary infection may be contributing factors for the intestinal lesions.[54] Remote deaths following x-ray irradiation are often related to infection, which may be in part attributed to leukopenia and diminished antibody formation [60] and in part to the dissolution of the epithelial defense barrier represented by the intestinal mucosa, which is extensively or completely destroyed.[61]

Lawrence and Tennant [62] have reported a study of the effects of x-rays and neutrons on mice, which furnishes an interesting parallel to our observations on $HN_2$ intoxication. Large doses induced death in 4 to 7 days, but at smaller doses deaths occurred up to 49 days. Females recovering were sterile, while males showed temporary sterility. Mice autopsied before 7 days showed lymphatic and bone marrow injury, and characteristic intestinal lesions, whereas animals dying after this period suffered continued weight loss, but at death various stages of bone marrow recovery were found. Blood cultures of mice dying before 4 days were sterile, but between 4 to 7 days they were occa-

sionally positive. Cultures obtained from mice dying after this period always contained bacteria, whereas they were sterile in sacrificed recovering mice. Lawrence and Tennant concluded that death following large doses of x-rays or neutrons is not due to infection but is related to "marked destructive changes in the various viscera mentioned above, giving rise to a toxemia from tissue breakdown products." As the dose is decreased, mice live longer, bacterial invasion is more likely, and infection comes to play a more important rôle. Chrom [61] believed that x-rayed mice die through a combination of intoxication, injury to the reticulo-endothelial system, and absence of defense against bacteria, so that finally fatal bacterial invasion occurs. Of interest in this connection is the demonstration by Osgood [63] that x-rays do not cause immediate cell death in cultures of bone marrow but interfere with mitotic activity, and the demonstration by Warren and Whipple [64] that chickens and pigeons are relatively resistant to radiation.

In general, then, lethal doses of x-ray appear to produce an extraordinarily close parallel to $HN_2$ intoxication, but this parallelism for the moment can be regarded only as superficial, since in neither case has the fundamental mode of injury or the ultimate cause of death been determined.

## Summary and Conclusions

1. The nitrogen and sulfur mustards are readily absorbed from the skin and mucosal surfaces, inducing injury to the lymphatic tissue, spleen, bone marrow, and the epithelium of the small intestine, and delayed death 3 to 6 days later.

2. The sequence of events at $LD_{50}$ doses consists of a relatively asymptomatic latent period of 1 to 2 days. During this time lymphatic injury is abrupt, the thymus, spleen, and lymph nodes involute rapidly, showing karyorrhexis, some karyolysis of the lymphocytes and depletion of these cells, phagocytosis of the débris, and a persistence and proliferation of epithelioid cells; at the same time there is a rapid reduction of the lymphocyte count in the peripheral blood. The hemopoietic cells of the bone marrow show injury, evidenced by changes in the staining reaction, vesiculation and fragmentation of nuclei, karyolysis, and nuclear alterations in the megakaryocytes. The epithelium of the small intestine shows vacuolization and nuclear swelling.

3. Following this period, anorexia, weight loss, and mucoid diarrhea ensue, and finally prostration and death occur in 72 to 144 hours. During this time lymphatic atrophy persists. The hemopoietic cells of the marrow disappear uniformly and the marrow becomes aplastic, consisting of dilated sinusoids, fat cells, and protein-rich fluid with a scattering of surviving cells, including megakaryocytes, reticular

and endosteal cells. The peripheral blood is severely leukopenic, due to a progressive fall in granulocytes. The red count falls slightly, but reticulocytes disappear, and it is evident that the production of hemopoietic cells has ceased. The small intestine is distended with fluid; and gastric stasis, possibly attributable to pyloric spasm, is consistently present in small animals. From the pylorus to the cecum the small intestine shows inflammatory and degenerative changes, with hyperemia, edema of the villi, sloughing of the epithelium, and metaplastic changes in the persisting and regenerating epithelium. Significant pathologic changes have not been observed in other tissues. The cause of these delayed deaths has not been determined.

4. Animals surviving $LD_{50}$ doses gradually recover weight and show restoration of bone marrow and lymphoid tissue and a return of leukocytes in the peripheral blood. The lymphocytes return rapidly, the granulocytes more slowly; and, as seen in the rabbit, recovery is characterized by a shift to the left, with the appearance of pseudo-eosinophilic polymorphonuclear leukocytes containing basophilic granulations, macropolycytes, agranular polycytes, basophils, and occasional abnormal red cells. The diarrhea subsides, and the intestinal epithelium is restored to normal.

5. Other animals show similar restoration of the bone marrow and leukocyte count, but continue to lose weight, with or without obvious secondary infection, and die at a remote period. These deaths do not appear to be directly related to the primary effects of $HN_2$.

6. Large doses parenterally induce rapidly appearing neurologic symptoms: convulsions, depression, incoordination, irritability, tremors, weakness, dyspnea, and parasympathomimetic activity. Death occurs in 1 to 40 hours, varying with dosage.

7. In mice, rats, and rabbits gassed at $LC_{50}$ concentrations most of the vapor is removed in the u[illegible]er respiratory tract and a considerable portion is absorbed to induce systemic intoxication, as evidenced by the usual hematologic and morphologic changes, and the occurrence of delayed deaths. Upper respiratory injury is severe and prolonged, and random deaths with continued weight loss and respiratory infection may occur at long periods after gassing. Large animals (dogs, cats, goats) suffer more severe pulmonary injury and die somewhat earlier at concentrations of the vapor possibly insufficient to induce lethal systemic intoxication.

8. When the hydrochloride is given orally, injury of the duodenal and jejunal mucosa with ulceration, hemorrhage, and sometimes perforation occurs; squamous metaplasia of the intestinal epithelium is a feature of healing. The presence of food in the gastrointestinal tract

appears to exert a local protective action. The compound may be absorbed from the intestinal tract to induce systemic intoxication.

9. The mammals which have been studied show no great differences in susceptibility, but chickens are relatively resistant to the intravenous injection of the hydrochloride, succumbing only to doses inducing an early neurologic effect (*i.e.*, they do not show the phenomenon of delayed death characteristic of mammals). Transient leukopenia occurs in the surviving birds.

10. For producing visible systemic injury in rats, H is superior at $LD_{50}$ doses to the β-chloroethyl vesicants ($HN_1$, $HN_2$, and $HN_3$), when administered intravenously or subcutaneously.

11. In a comparative study of the delayed systemic action of H, $HN_1$, $HN_2$, and $HN_3$, rats were exposed to these compounds via cutaneous application, gassing, intravenous and subcutaneous injection at dosage in the range of the $LD_{50}$ or $LC_{50}$, and about 0.5 $LD_{50}$ or $LC_{50}$.

a. Wide variations occur between the effects obtained by the *intravenous* and *subcutaneous* routes and those following cutaneous application or gassing.

b. After *gassing* and *percutaneous* application the systemic effects of HN compounds, at both levels of dosage, were more severe than after H, with the single exception that $HN_1$ was less myelotoxic than the other three compounds. On the basis of dosage required, $HN_3$ was the most efficient in producing the delayed systemic effects.

c. *Intravenous* and *subcutaneous* injection of H (in propylene glycol) reverses the relationship to the HN compounds; at the $LD_{50}$ dosage, it was most consistent and toxic in its action judging by the loss of weight, leukopenia, extent of lymphoid atrophy, bone marrow injury, and enteritis. $HN_3$ was again the most efficient of the nitrogen mustards; $HN_1$ was the least leukotoxic at the dosage employed.

12. Comparison of the delayed systemic effect of nitrogen and sulfur mustards with other leukopenic agents (benzene and x-rays) shows them to be remarkably similar to x-rays (including the enterotoxic action), whereas parallelism to benzene is less evident.

For an interesting comprehensive and quantitative study of the effects of single intravenous injections of the same nitrogen and sulfur mustards at doses approximately equal to our $LD_{50}$ dosage, the reader is referred to the paper by Kindred, J. E., Histologic changes occurring in the hemopoietic organs of albino rats after single injections of 2-chloroethyl vesicants. *Arch. Path.*, 1947, **43**, 253–295, which appeared after our paper had been submitted for publication. Kindred's general observations are in essential agreement with ours.

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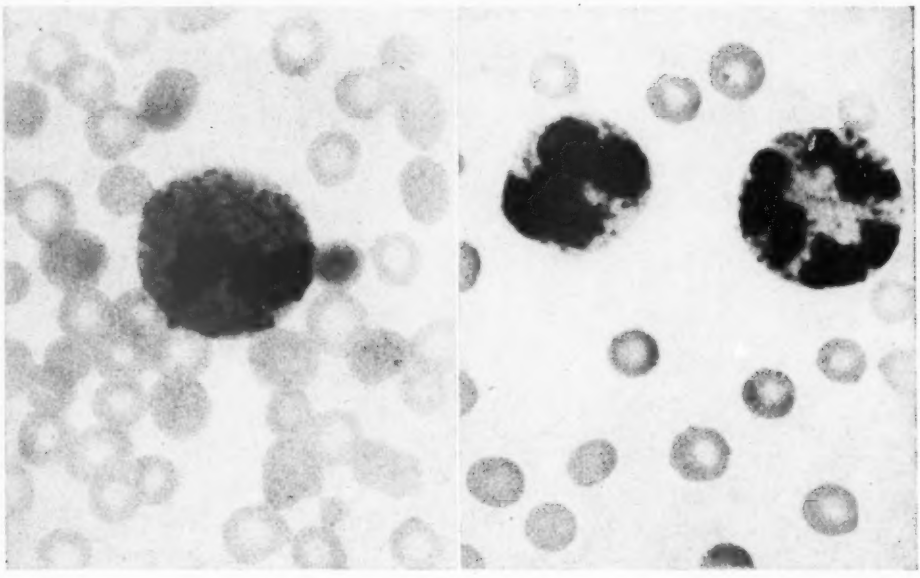
[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

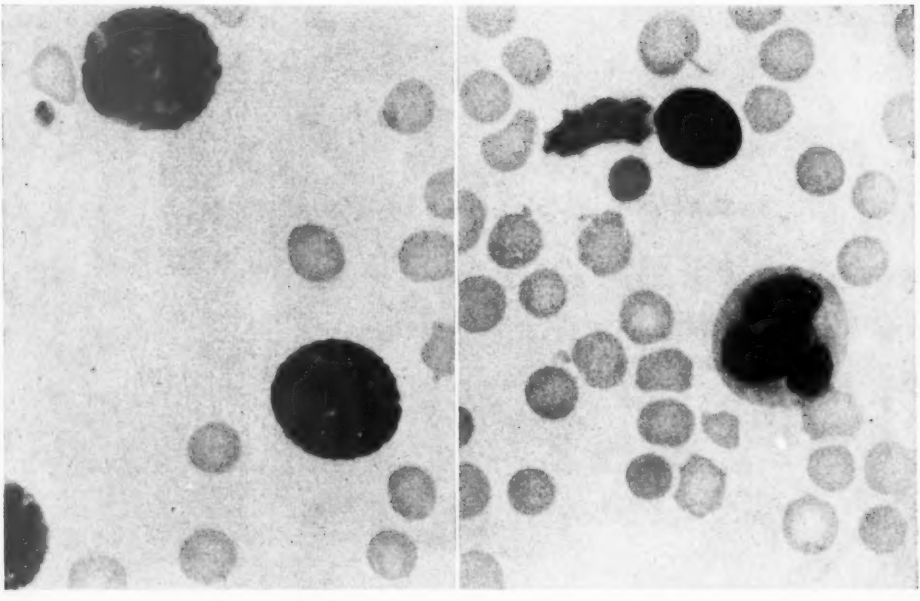
### PLATE 1

FIG. 1. Polymorphonuclear leukocytes which may occur in rabbit blood during the recovery phase. These cells first appear from 4 to 5 days after administration of 0.5 to 3.0 mg. per kg. of $HN_2$ hydrochloride and may persist for 2 weeks thereafter.

A. Macropolycyte: nucleus in mitosis, basophilic granulations. B. Pseudo-eosinophilic polymorphonuclear leukocytes: cell on the right shows an excessively segmented nucleus. C. Pseudo-eosinophilic polymorphonuclear leukocytes: basophilic granulations. (This is a constant and the most common form of abnormal cells.) D. Lymphocyte and an agranular pseudo-eosinophilic polymorphonuclear leukocyte.



1-A 1-B



1-C 1-D

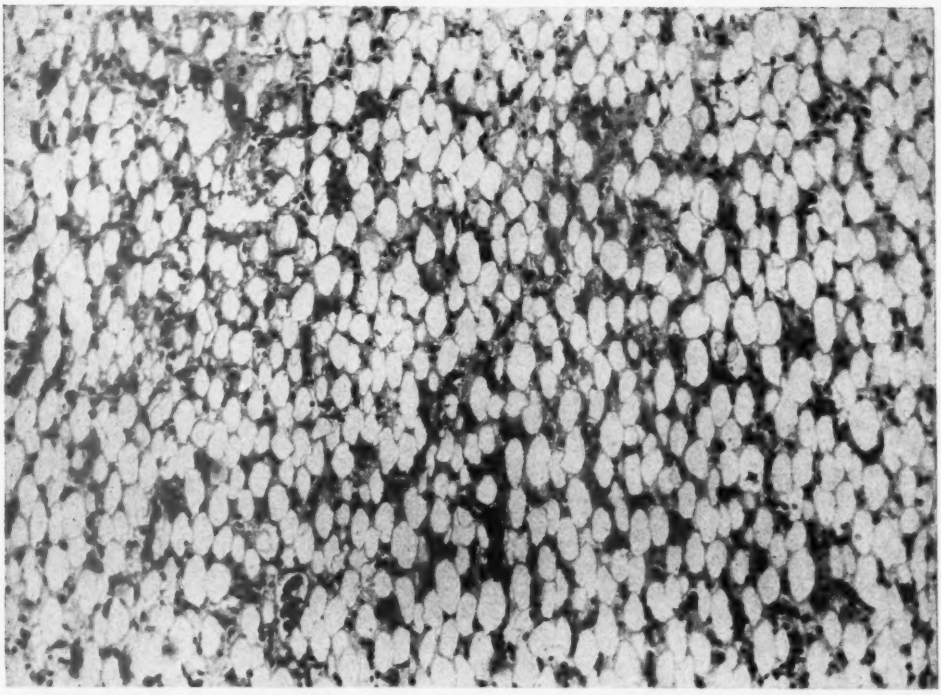
## PLATE 2

FIGS. 2 and 3. Femoral (Fig. 2) and sternal (Fig. 3) bone marrow of a rabbit given 3.0 mg. per kg. of $HN_2$ HCl intravenously and sacrificed 70 hours later. The photomicrographs illustrate the severe depletion of cells and the persistence of fat and an irregular deposit of protein-containing fluid in the interstitium. Figure 2, × 170; Figure 3, × 130.

2

3

2



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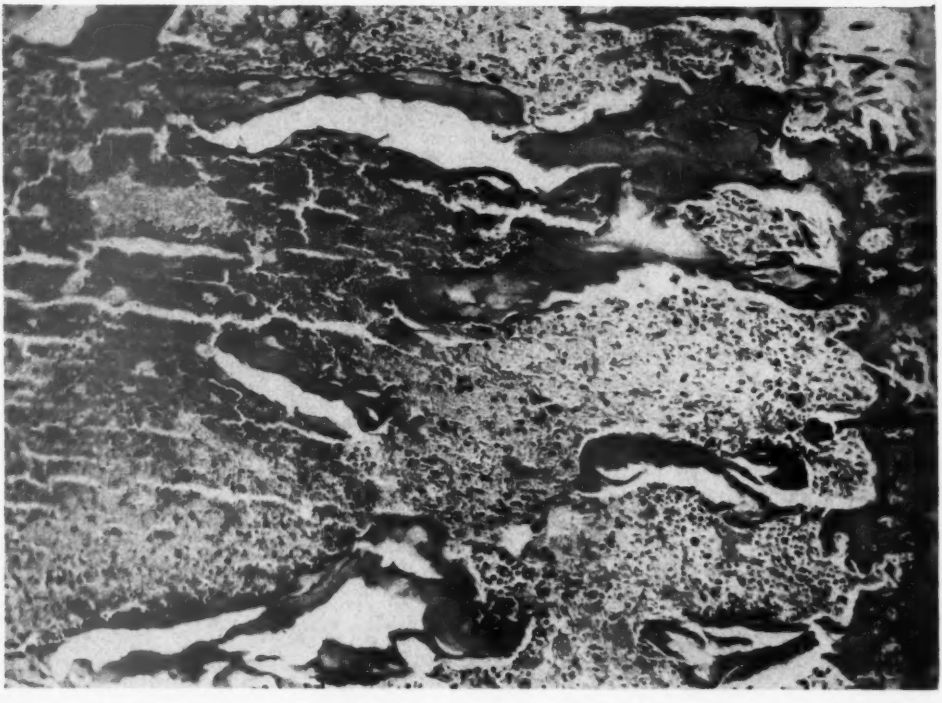


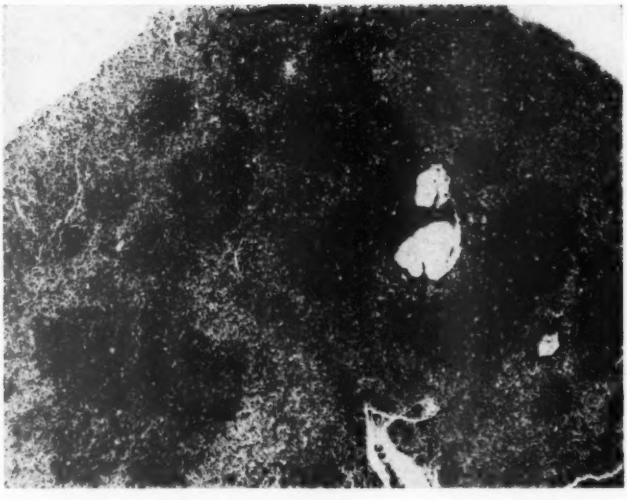
Graef, Karnofsky, Jager, Krichesky, and Smith Effects of Nitrogen and Sulfur Mustards

PLATE 3

FIG. 4. Low-power photomicrograph illustrating splenic contraction in a rat 72 hours after gassing for 10 minutes at 0.4 mg. per liter. The follicles are about one-half their usual size, and the red pulp is represented by fibrous strands and sinuses engorged with erythrocytes. Hematoxylin and eosin stain. × 30.

FIG. 5. Low-power photomicrograph illustrating thymic involution in a mouse 70 hours after receiving 16 mg. per kg. of $HN_2$ subcutaneously. Of note are the great reduction in lymphocytes of the cortex and the replacement by large epithelioid cells. Lymphocytes persist in the medulla. The pattern suggests a reversal of the normal corticomedullary composition. Hematoxylin and eosin stain. × 30.

4



5

PLATE 4

FIG. 6. Photomicrographs of sections of the jejunum of rats.

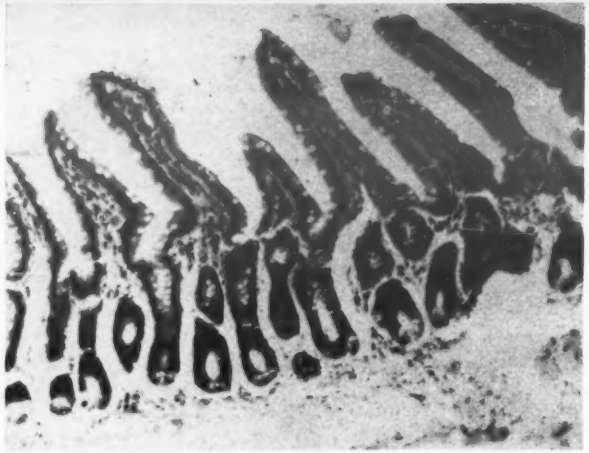
A (× 150) illustrates the villi and normal appearance of the epithelium in an untreated animal (apparently free from paratyphoid infection or parasitic enteritis). B and D illustrate the enterotoxic effect of $HN_2$ hydrochloride at 48 hours (3 mg. per kg. subcutaneously) and C, at 96 hours (2 mg. per kg. intravenously). All sections were stained with hematoxylin and eosin.

In B, the villi are broader than normal and shorter. Subepithelial edema has lifted the lining cells of the villi. Deep in the mucosa, dilated lymphatics may be seen. These often contain precipitated protein-rich fluid. There is an increase of cells, chiefly eosinophilic leukocytes and some polynuclear leukocytes. × 100.

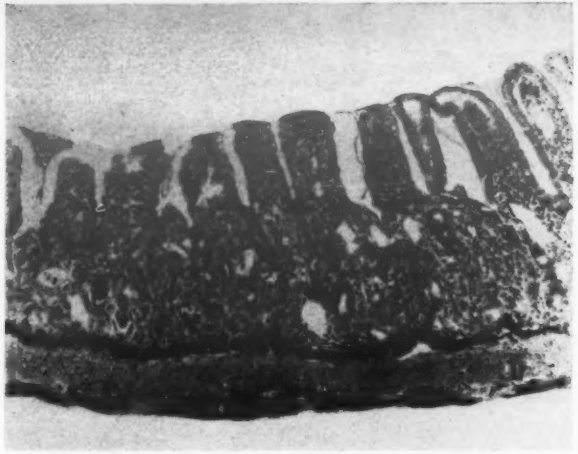
In C, a later stage is depicted. Of note are the striking vesiculation of the cytoplasm of the surface cells and evidence of desquamation. The villi are short and abut on each other. In the crypts of Lieberkühn, squamous metaplasia has taken place and the cells of Paneth are no longer seen. The cellular reaction in the interstitium is not marked or in excess of normal findings, as is often the case. × 250.

In D, the villi are short, stubby, and the mucous epithelium is partially replaced by hypertrophic and hyperchromatic cells with large nuclei. In places, the epithelium is low-columnar; in others, it is of a squamous character. In one crypt is a large plug of detritus including nuclear débris and some polynuclear leukocytes. × 250.

6-A



6-B



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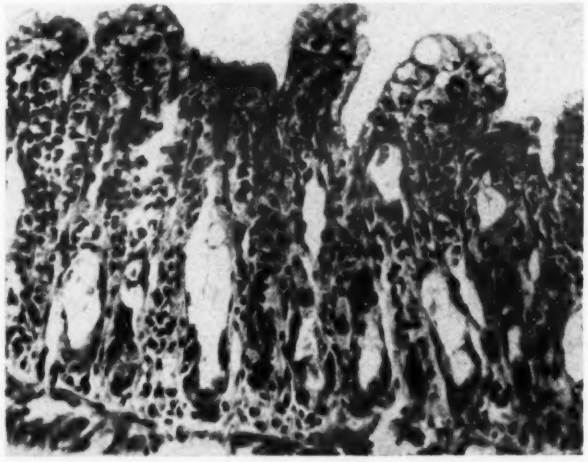
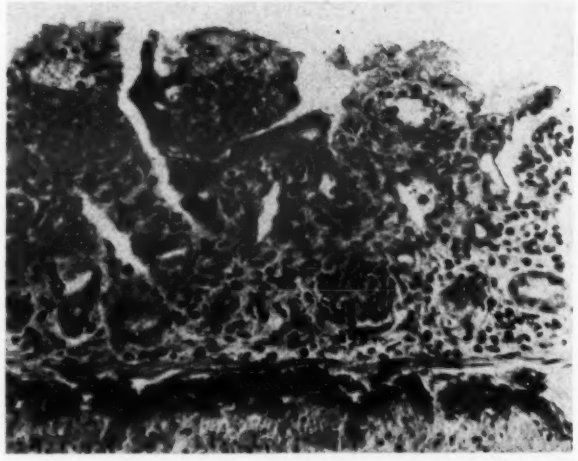


6-D



Graef, Karnofsky, Jager, Krichesky, and Smith

Effects of Nitrogen and Sulfur Mustards

# SOME ASPECTS OF THE EVOLUTION OF SILICOTIC LESIONS*

I. Costero, M.D.

*(From the Department of Pathology, University of Mexico School of Medicine, and the Instituto Nacional de Cardiologia, Mexico, D.F.)*

The microscopic appearance of silicotic lesions is well known today and may be found described in the standard textbooks on pathology. These descriptions are based on the classical staining technics with aniline dyes. I have studied 35 cases of pulmonary silicosis with the silver impregnation technics of Río-Hortega and have obtained a number of new or little known structural details which may contribute to a better understanding of the evolution of the silicotic nodule and which explain the presence of those other non-nodular lesions that are constantly observed in the lungs of patients with silicosis.

Silicotic lesions are not due exclusively to the direct effect of silica dust inhaled into the alveoli but are influenced also by secondary infections. However, these infections, especially tuberculosis, are present so constantly with silicosis that it becomes difficult to trace a sharp line between lesions which are purely silicotic and those which are mixed; therefore, they are being described together.

## Technical Methods

The staining methods employed in this report are described in detail in the publications of P. del Rio-Hortega, which are listed in the bibliography.† The following is a summary of those methods which gave the best results in staining silicotic nodules.

### *Río-Hortega's Method for Precollagen Fibers*

1. Fix in 10% aqueous solution of formalin (indefinite time).
2. Cut sections on the freezing microtome.
3. Wash in distilled water (15 minutes).
4. Immerse in a recently prepared 5% aqueous solution of potassium permanganate; each section must remain in this solution a different period of time, *i.e.*, 15, 30, and 60 seconds, and 2, 4, 8, and 16 minutes; thus seven sections will be necessary.
5. Wash rapidly in distilled water (5 to 15 seconds).
6. Remove the potassium permanganate retained in the sections with a 5% aqueous solution of oxalic acid (15 seconds).
7. Wash rapidly in distilled water (5 to 15 seconds).
8. Wash in ammoniacal water: distilled water, 50 cc.; ammonium hydroxide, 1 drop (5 to 15 seconds).
9. The seven sections which have followed all the steps, and another two not

* Received for publication, January 31, 1947.

Cost of illustrations partially defrayed by the Committee on Inter-American Scientific Publications.

† These publications may be obtained in the Institución Cultural Española, Bernardo de Irigoyen 672, Buenos Aires, República Argentina.

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previously treated with permanganate, are placed in a cylindrical dish of about 10 cc. capacity with a 1.5% aqueous solution of silver nitrate; heat the solution until the sections become a yellowish color (about 30 minutes at 60°C.); stir frequently.

10. Wash rapidly in distilled water (5 to 15 seconds).

11. Immerse the sections in another cylindrical dish in Río-Hortega's silver carbonate:

10% aqueous solution of silver nitrate ..... 10 cc.
5% aqueous solution of anhydrous sodium carbonate ..... 40 cc.

The precipitate formed when both solutions are mixed is dissolved with ammonium hydroxide; this must be added drop by drop very carefully; decant when a few particles of precipitate still remain (the excess of ammonium hydroxide must be avoided). Add distilled water to make 150 cc.

Before immersing the sections in Río-Hortega's silver carbonate, add 3 drops of pyridine (if the dish is of 10 cc. capacity). Cover the dish with a watch-glass and heat slowly until the sections become of a blended tobacco-like color (about 20 minutes at 60°C.); stir frequently.

12. Wash rapidly in distilled water (5 to 15 seconds).

13. Reduce in 10% aqueous solution of formalin; the sections are rapidly obscured (black tobacco-like color, which appears in 15 seconds).

14. Wash in distilled water (1 to 15 seconds).

15. Immerse in a 0.2% aqueous solution of gold chloride at room temperature; stirring frequently until the black tobacco-like color changes into gray tones (15 minutes); then heat 10 minutes at 40°C.

16. Fix in a 5% aqueous solution of sodium thiosulfate (1 minute).

Wash in distilled water; dehydrate in 96% alcohol; clear in beechwood creosote; mount in Canada balsam.

Results: The cells and the connective stroma are simultaneously stained. The longer the time in the permanganate solution, the paler the color of the cells and the clearer the contrast of the connective stroma. The precollagen fibrils are stained in black tones, while the collagen bundles are of purplish color.

## *Río-Hortega's Method for Macrophages*

1. Fix in 10% formalin (maximum, 1 month).

2. Cut sections on the freezing microtome.

3. Wash in distilled water (10 to 20 minutes). When the specimen has been fixed for more than 10 days and less than 1 month, it is convenient to leave the sections for 24 hours in distilled water, and even better in a diluted solution of sodium sulfite or in ammoniacal water.

4. Immerse one section in Río-Hortega's silver carbonate for 5 seconds, stirring continuously in order to obtain a homogenous impregnation; change immediately to a 1% aqueous solution of formalin. The results will be different whether the formalin is stirred or not, and both possibilities should always be tested; the sections will be reduced in less than 1 minute if the solution is stirred, but they will need 5 or more minutes for reducing if the solution remains without movement.

A second section is immersed for twice as long in the silver carbonate, and the reduction is made in the same manner. The other sections are left in the silver carbonate for increasing periods of time, doubling the number of seconds with each, the limit being 15 minutes.

After staining, the complete series of sections must be observed under the microscope; the optimum time for the staining of macrophages, which is variable for each specimen, may be determined without mounting the sections and even using low magnification.

5. Wash in distilled water (indefinite time).
6. Tone in the solution of gold chloride.
7. Fix in the solution of sodium thiosulfate.
8. Wash in distilled water. Other complementary staining may be advantageously used at this moment, depending on the structures that are to be observed simultaneously with the macrophages, such as nuclei, fat, hematogenic pigments, etc.

Dehydrate, clear and mount.

Steps 6, 7, and 10 are identical with 15, 16, and 17 of the method for precollagen fibers.

Results: The cytoplasm of the macrophages is stained in different intensities of purplish color; all of the other structures are weakly stained or invisible, if complementary stains are not used.

### *Gallego's Method for Elastic Fibers*

1. Fix in 10% formalin (indefinite time).
2. Cut sections on the freezing microtome.
3. Place the sections in a Petri dish containing about 40 cc. of water; they should be stained promptly, because the water removes the rest of the formalin, some of which must remain in the sections, in order to obtain perfect staining.
4. Immerse the sections for 5 minutes in:

| | |
|---|---|
| Water | 10 cc. |
| Pharmacopoeial solution of ferric chloride ($FeCl_3 + 6H_2O$) | 5 drops |
| Nitric acid | 1 drop |

5. Transfer sections, without washing, for 5 minutes, to:

| | |
|---|---|
| Water | 10 cc. |
| Ziehl-Neelsen's carbolfuchsin | 30 drops |
| Nitric acid | 1 drop |

This solution and the former (4) must be prepared immediately before using.

6. Wash rapidly in water (5 seconds).
7. The sections are differentiated during 5 minutes in the same solution used in step 4.
8. Wash in water. The sections may be observed under the microscope at a low magnification; the elastic fibers must be the only structures which appear deeply stained. If the staining is pale, steps 4, 5, 6, and 7 may be repeated once or twice.

Dehydrate in alcohol, clear in beechwood creosote, wash out the creosote with xylene, and mount in Canada balsam.

A beautiful and complete counterstain may be obtained by adding the following complementary steps:

9. Immerse the sections for 2 minutes in:

| | |
|---|---|
| Water | 10 cc. |
| Ziehl-Neelsen's carbolfuchsin | 10 drops |
| Acetic acid | 1 drop |

10. Wash rapidly in water (5 seconds).
11. Immerse the sections for 5 minutes in:

| | |
|---|---|
| 1% aqueous solution of formalin | 10 cc. |
| Acetic acid | 1 drop |

12. Wash in water (5 to 15 minutes).
13. Stain in Cajal's picro-indigo carmine solution (1 minute):

| | |
|---|---|
| Aqueous saturated solution of picric acid | 90 cc. |
| Distilled water | 10 cc. |
| Indigo carmine | 0.25 gm. |

14. Wash in water (15 seconds).

Dehydrate and mount as before (8).

Results: Elastic fibers, the nuclei, and all basophilic structures appear stained in different tones of violet; the collagen bundles and the acidophilic structures appear blue; the red blood cells and the muscle fibers are a yellowish green.

## Histogenesis and Evolution of the Typical Silicotic Nodule

According to my observation, the initiation of the nodules takes place around vessels which are reached by the minute sharp silica crystals carried by the alveolar macrophages. At first the process is marked by a proliferation of the reticular (precollagenous, argyrophilic) fibers which appear to form a moderately dense net sustained by a number of thick trabeculae of radiating disposition, which in turn are anchored in a few circular collagenous bands surrounding the central vessel (Fig. 1). This lesion is usually well limited, but the proliferation may extend into the neighboring interalveolar septa. There are always great numbers of desquamated cells of macrophagic character in the adjacent alveoli, and small quantities of albuminous exudate may be present.

While the reticular fibers continue to proliferate rapidly, the central vessel also becomes involved, at first showing signs of obliterating angiitis and finally becoming completely obstructed. The argyrophilic fibers lose their reticular arrangement, become wavy, and are partially transformed into collagenous substance. Through this process there arises a spheroid nodule which stands out clearly from the neighboring alveoli by its hardness and lack of vascularity (Fig. 2).

The greater the number of newly formed collagenous (fascicular, acidophilic) fibers, the slower the further growth of the nodule. When retraction of these collagenous fibers ensues, it causes compression atelectasis of the parenchyma with collapse of the surrounding vessels and bronchi, and there appears a desquamation of alveolar cells of variable intensity. The static period is reached when all of the reticular fibers of the nodule have been replaced by collagenous fibers. Figure 3 shows a large-sized nodule which is still growing (as demonstrated by the persistence of reticular structure), and in it one may observe the intensity of the desquamative process and the widening of the lymphatic spaces. Figure 4 shows a node in which the growth has been arrested; it is made up exclusively of thick collagenous fibers. Here the limiting desquamative process has become organized and static, and the lymphatic spaces are no longer conspicuous.

The first precollagenous fibers to appear in the lesions grow out from the adventitial layer of the small vessels, especially from the venules which have been attacked by silica crystals accumulating in their lymphatic spaces; the subsequent growth of these fibers then becomes autonomous. Other new fibers are formed by the regional histocytes

situated, in order of importance, within the adventitia of small vessels, in the local capillaries, and in the alveolar wall itself.

### Factors Causing the Polymorphism of the Silicotic Node

In only a few cases is the structure of the silicotic nodule as simple as in the foregoing description since it is subject to modification by many factors.

Several incipient nodules may be so close to one another that their proliferating reticular fibers coalesce and they become fused into the likeness of acini or clover leaves (Fig. 5). This image is probably the result of the simultaneous proliferation around several vessels joining two main nodules, each one of them composed of smaller secondary ones. New nodules sometimes appear near the border of other more advanced lesions. In Figure 6 there are two such nodules incompletely separated from an older one by a few collapsed alveoli. This mechanism of spreading of the silicotic nodule may be termed *growth by apposition.*

There is not always as sharp a limit between the older nodule and its satellites as in the foregoing example. Figure 7 shows the edge of a silicotic nodule still in the precollagenous stage which grows outward irregularly through the formation of secondary nodules, with multiplication of fibers alongside the vessels and invasion of the alveoli. Such formations may indicate a continuous arrival of siliceous dust in the alveoli and its accumulation around the vessels irrigating the border of a nodule.

Another factor contributing to the progression of the lesions is the limiting desquamative pneumonia which is a constant companion of spreading silicotic nodes. Figure 8 illustrates such a process by showing the marginal alveoli of a nodule filled with desquamated cells and scanty albuminous exudate of vascular origin. The relative integrity of the interalveolar septa is useful in differentiating this aseptic irritative process from lesions caused by either banal or tuberculous infection.

With adequate technics and high magnifications the desquamated cells are shown to have pseudopodial forms, which indicate active migration and, therefore, macrophagic character (Fig. 9). The exudate itself is often free of blood corpuscles. The small, round, often pyknotic nuclei, and the products of cytorrhexis which appear in the photomicrograph belong to desquamated cells and only exceptionally to leukocytes or lymphocytes.

Desquamative pneumonia contributes to the spread of silicotic lesions because its exudate tends to become organized. Figure 10 shows the delicate reticular fibers largely originating in the wall of the alveoli and partly formed by the desquamated histocytes. As they replace the

intra-alveolar fluid there appears a reticulum similar to the structure of the neighboring nodes; the new reticulum slowly coalesces with the older nodes as shown in Figure 11. In some regions where the alveolar wall is still preserved the new reticulum is quite definite, but in other sites the boundary between the silicotic nodes and the organized exudate has disappeared. This second type of growth may be defined as *growth by organizing pneumonitis*.

The third and last factor causing polymorphism of the lesions is the presence of atelectatic areas around the nodes. If compression were the only cause of atelectasia, one should not expect the latter to contribute to the growth of the lesions, but there are also inflammatory phenomena due chiefly to the presence of siliceous dust in the alveoli and to the ischemia resulting from the compression of the blood vessels. These inflammatory phenomena also contribute to the early destruction of the alveolar epithelium and thus break down the barrier between the reticular fibers of the interalveolar septa and the lumina of the alveoli which contain the irritative factor. This breakdown starts proliferation of the fibers. Finally, fibrils from opposing walls become fused and a solid mass arises which comes into contact with neighboring nodes as shown in Figure 12. This *growth by transformation of atelectatic zones* can be distinguished from both of the previous types of growth by one significant detail: whereas in growth by apposition and in growth by organizing pneumonitis the structure of the node is predominantly reticular, collagen transformation must have taken place in order to produce adequate zones of atelectasia.

## Observations on the Structure of the Silicotic Node

Silver impregnation technics have revealed details referring mainly to the intranodular blood vessels, the connective fibrils, the process of central softening, and to the activity of the macrophages.

The most frequent change in the *intranodular blood vessels* is the widening of the adventitial lymphatic space. In Figure 13 the central vessel of the node has been cut longitudinally and the endothelial and muscular layers show little change, while the perivascular lymphatic space appears as a wide channel conspicuous by its net of reticular fibers. Figure 14 shows a similar vessel cut transversely. Within the dilated space there may be small numbers of lymphocytes, macrophages, and silica crystals. I believe that these elements may be carried there passively following the physiologic draining current from the alveoli.

Alterations of the vascular intima begin somewhat later. In Figure 14 there is already a slight proliferation of the endothelium with conse-

quent narrowing of the lumen. Figure 15 illustrates the period of maximal proliferation and shows the lumen completely obliterated. This vascular obliteration may be responsible for the transformation of precollagen fibrils into collagen, to be discussed later in this paper.

The degenerative changes of intranodular vessels are often compensated by a new growth of capillaries originating from vessels situated outside of the lesion. The new capillaries may have to traverse wide distances and they behave similarly to, but not exactly like, capillaries arising in canalizing thrombi or in foreign body reactions. They are difficult to demonstrate with aniline stains but appear clearly when silver impregnations are used, as in Figure 16 where they appear as delicate tubes of precollagen fibrils. Since this section is very thin, only a minority of these newly formed capillaries can be discerned. Their real number is more apparent when retraction takes place and the density of the structures increases, as in Figure 17 in which the nodule is invaded by a new net of capillaries penetrating it from outside. The relation between this process and the softening of the silicotic nodule will be discussed later.

The *structure of the reticular network* of the silicotic node is quite variable. When the transformation into collagen has progressed but little, the argyrophilic fibers preserve their characteristic reticular aspect (Fig. 18); the radiating disposition previously described is often very clear. Sometimes at the border of the growing nodule, sometimes in its center and near the radiating zones, the fibers become more independent and show a tendency to form wavy bundles; this indicates the first change in their chemical composition, also shown by their avidity for acid dyes. Soon after, they break up into parallel bundles and acquire the histologic characteristics of collagen fibers (Fig. 19). The number of straight reticular bundles increases and it is common to find nodules like that in Figure 20, which consist of fibers of trabecular disposition but which otherwise conserve their precollagen characteristics. One must be careful to distinguish these bundles of wavy precollagen fibers, which mark the beginning of collagenous transformation, from those entirely straight fibers which are the result of mechanical traction (Fig. 21). The preponderantly parallel arrangement here is due to the displacement resulting from the retraction of the older collagen bundles in the neighboring nodules.

In Figure 22 are found the first collagen bundles arising from the wavy fibrils in the center of a silicotic nodule. Here also, as occurs usually when collagen is formed by an abnormal mechanism, the fibers soon become hyalinized and contracted. This causes the whiteness and hardness of the nodules that characterize their macroscopic appearance.

However, there are also autolytic phenomena which tend to produce the opposite result. The softening of completely fibrous nodes is due in part to the formation of new capillaries and in part to the proteolytic action of the phagocytes contained in the lesions. Figure 23 is taken from such a softening nodule; in the loose zone the central vessel and débris of dissolving reticular fibers may be observed. In Figure 24 the central area is filled with lymphoid cells and the penetration point of the vessels which have produced the softening can be distinguished in the lower part of the figure. In Figure 25 there are numerous active macrophages beside the vessels and lymphocytes. The last three illustrations taken together show the main features of softening in successive stages.

Among the many elements found in softening foci, cellular débris, masses of siliceous crystals, and fragmented fibrils can be observed. There are also some elements whose significance and mode of origin are not clear, as, for instance, the chrysophilic granules shown in Figure 26; they have irregular shapes with generally rounded contours and they stain deeply with warm gold chloride. Their appearance would have suggested derivation from erythrocytes but for the lack of any other sign of hemorrhage in the softened silicotic nodules. Another problematic feature concerns the large refringent crystals with indefinite edges (Fig. 27) which are occasionally present. They show varying degrees of affinity for the basic aniline dyes, as do calcium salts, and they are found in the vicinity of other basophilic granules. They resist treatment with both alcohol and xylene.

Study of the *macrophages* with the specific silver impregnation technics of Río-Hortega has contributed much valuable data about the histogenesis and evolution of the silicotic nodule. Macrophages appear quite early surrounding the vessels of a developing nodule (Fig. 28). Many of them come from the alveoli and transport the siliceous crystals into the adventitial spaces where the silicotic lesions begin, but not all of the crystals arrive in the adventitial space in this manner and probably a considerable number of the perivascular macrophages are formed *in situ* by the histocytes surrounding the altered venules. When the irritation by the dust particles excites the reticular fibers to proliferate, the adventitial space soon becomes obliterated; thus, the irritating dust particles are accumulated around the vessels where they stimulate the formation of more macrophages. The number of macrophages increases progressively until there are large masses of ameboid cells containing cellular detritus (Fig. 29).

The detailed structure of the macrophages can be observed in Figure 30, taken at a higher magnification. The cytoplasm is highly developed

and shows rounded shapes. The nuclei are hidden by the numerous intracellular inclusions, including siliceous crystals. Sometimes there are small vacuoles that give the microchemical reactions of the fatty substances and some of the pseudopodial prolongations are found detached from the main body of the cells.

When the reticular fibers are transformed into collagen there begins a rapid destruction of the macrophages. Some of them are caught between the meshes of the hyalinizing bundles during their process of hardening and retraction and space becomes too limited even for cells as stereotropic as these. Figure 31 shows the macrophages imprisoned between the thick hyalinized collagen bundles. However, the greater part of the destruction takes place in the softened areas by autolytic changes (Fig. 32); the intensity and speed of the regressive phenomena can be deduced from the pyknotic nuclei and liquefying cell bodies.

The previously well known fact of the disappearance of the elastic fibers has been further confirmed by my observations. Figure 33 from a preparation stained by Gallego's method shows the absence of elastic fibers in the nodular zone and in addition their radiating orientation towards the periphery of the nodule as a result of the traction exercised by the shrinkage of the newly formed collagen fibers.

## Diffuse Silicotic Lesions

Fibrous nodules are the most characteristic type of lesion in silicosis, but other types are present. Nodules are frequently found together with diffuse fibrous plaques, peribronchial sclerosis, and with focal pleural thickening.

*Diffuse fibrous plaques* may be interpreted as nodules profoundly modified by vascular proliferation, migration of macrophages, and superimposed inflammatory lesions (Fig. 34). Among the inflammations, tuberculosis apparently plays the most active part in the transformation of lesions into extreme fibrous plaques. The collagenous network of these plaques is so irregular that retraction has few consequences and there is only slight deformation of alveoli, vessels, and bronchi.

*Peribronchial sclerosis* is probably a product of the same factors which excite the formation of the nodules, namely, the penetration of particles of silica into the lymphatic spaces; but, since the efficiency of lymphatic drainage is lower in the vicinity of the bronchi and there is reticular connective tissue within their mucosa, well defined nodules are the exception rather than the rule (Fig. 35). There is an intense desquamation of the mucosa and the lumen of the bronchi contains exudate, although it must be noted that these phenomena are exaggerated by post-mortem autolysis due to delay in fixing the specimen.

The *pleuritis* of silicosis often appears rather early, sometimes being the first anatomic change of the disease. It begins by a moderate thickening of the visceral pleura with considerable dilation of its lymphatics and blood vessels. The hypertrophy of the connective structure is relatively slow and usually the reticular network, which characterizes the parenchymal lesions of silicosis, fails to develop. The neighboring alveoli are not affected until late in the process (Fig. 36).

When pleural thickening has reached full development, as in Figure 37, the surface of the serous membrane is found covered with newly formed capillaries together with a delicate network of reticular fibers. These fibers are formed by wandering histocytes, many of which show the changes of fibrogenesis. The vessels, especially the lymphatics, are always dilated. The lesions show a tendency to limit themselves to plaques which can be observed macroscopically; there is either very little hyalinizing of the collagen fibers or none at all. The presence of silicotic nodules in the immediate vicinity of the pleural plaques seems to increase the size of the latter. It is to be assumed that the pleural lesions have the same significance as the perivascular nodules; they are also a reaction to the presence of siliceous crystals, either free or carried by macrophages, arriving through the lymphatic stream.

## Summary

After studying the structure and evolution of the silicotic node with the silver impregnation methods of Río-Hortega, the following conclusions have been reached.

1. The histologic basis of all lesions induced in the human lung by siliceous dust is the proliferation of the reticular fibers. After proliferation these fibers are partially or wholly transformed into collagen and undergo hyalinization and retraction.

2. The silicotic node continues its growth as long as there are reticular fibers left; growth is arrested when collagenization is complete.

3. The reticular fibers of the nodule originate mainly in the adventitial layer of small vessels, especially the venules which have been attacked by siliceous dust deposited in their adventitial lymphatic spaces. Fibrogenesis from regional histocytes is of secondary importance.

4. The nodules grow by (a) apposition of adjacent nodules in the later stages of development, (b) organization of the exudate of the desquamative pneumonia which marks the limit of the developing nodules, and (c) transformation of adjoining atelectatic zones.

5. The adventitial lymphatic space of the blood vessels is frequently found dilated and obstructed by proliferating reticular fibers. Sometimes the nodes are penetrated by newly formed capillaries arising from nearby interalveolar septa.

6. Many of the silicotic nodules undergo a softening process due to the penetration of newly formed capillaries and to the autolytic action of macrophages. In the softening foci I have found chrysophilic masses and basophilic crystals whose origin and significance cannot be interpreted at this moment.

7. The macrophages found in silicotic lesions are first seen around the vessels, shortly after the beginning of the proliferation of reticular fibers. They contain great amounts of detritus and siliceous particles. They are destroyed later when they are compressed by bundles of hyalinizing collagen and also in great part by autolysis.

8. The elastic fibers disappear in the completely constituted lesions. Their disposition outside the latter is indicative of the traction originated by the shrinking of silicotic nodules in the stage of collagenous transformation.

9. Beside the typical silicotic nodule there also appear diffuse sclerotic plaques, peribronchial sclerosis, and proliferative pleuritis. These three lesions have special characteristics which can be related to the different types of lymphatic drainage pertaining to each region; however, their histogenesis, structure, and evolution are similar to those of the typical silicotic nodule.

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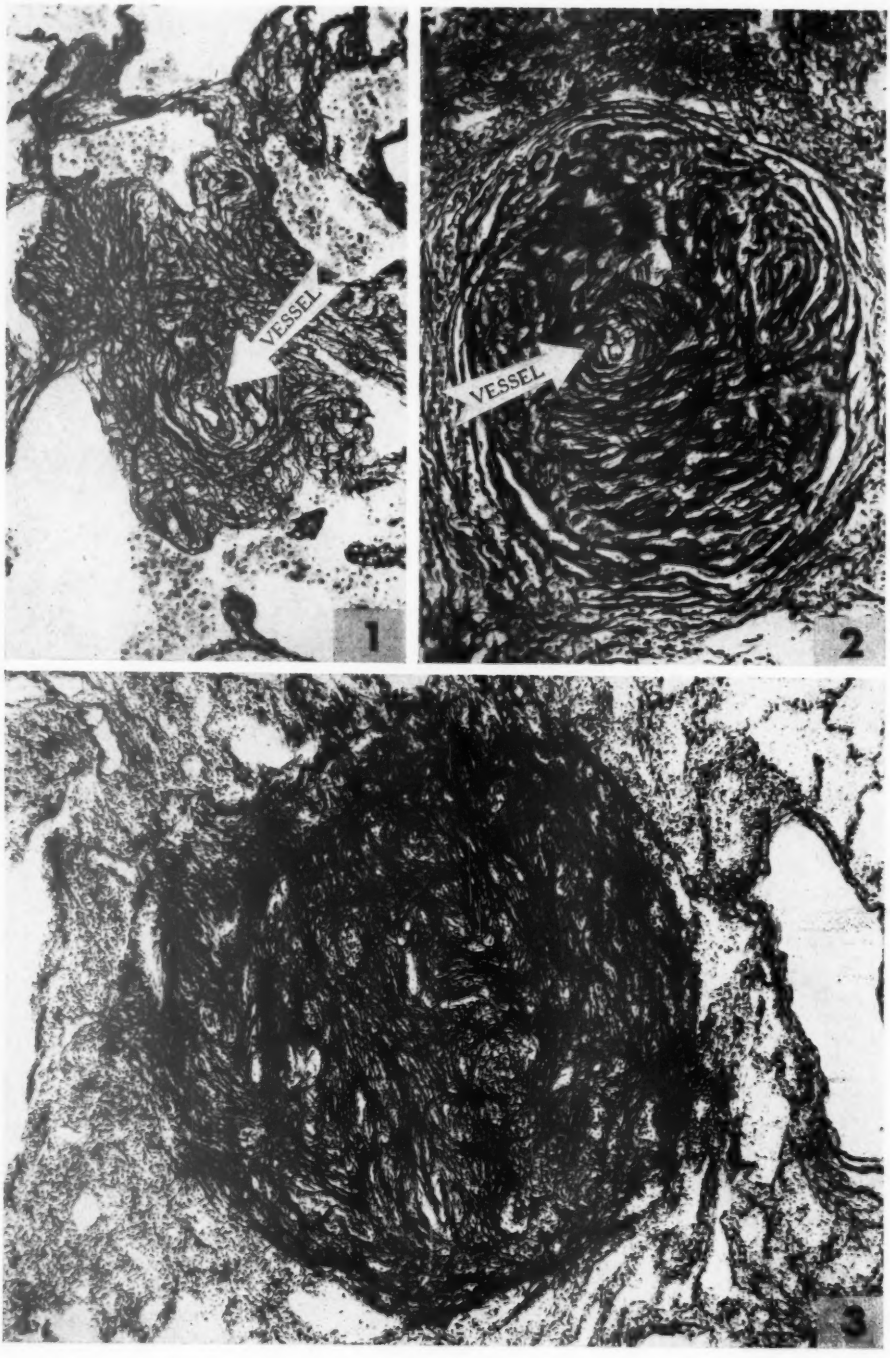
## DESCRIPTION OF PLATES

### Plate 5

Fig. 1. Silicotic nodule in its initial stage, showing proliferation of the reticular fibers around a vessel. The lumina of the alveoli contain great numbers of desquamated cells and some albuminous exudate. × 110.

Fig. 2. Silicotic nodule in evolution. The central vessel is obliterated. The connective fibers are no longer arranged in reticular form but are in bundles and are partially transformed into collagen. × 110.

Fig. 3. Silicotic nodule of greater size than that shown in Figure 2 but still consisting mainly of reticular fibers. The desquamative pneumonitis in its periphery and the dilatation of lymphatic spaces indicate the evolutive character of the lesion. × 70.

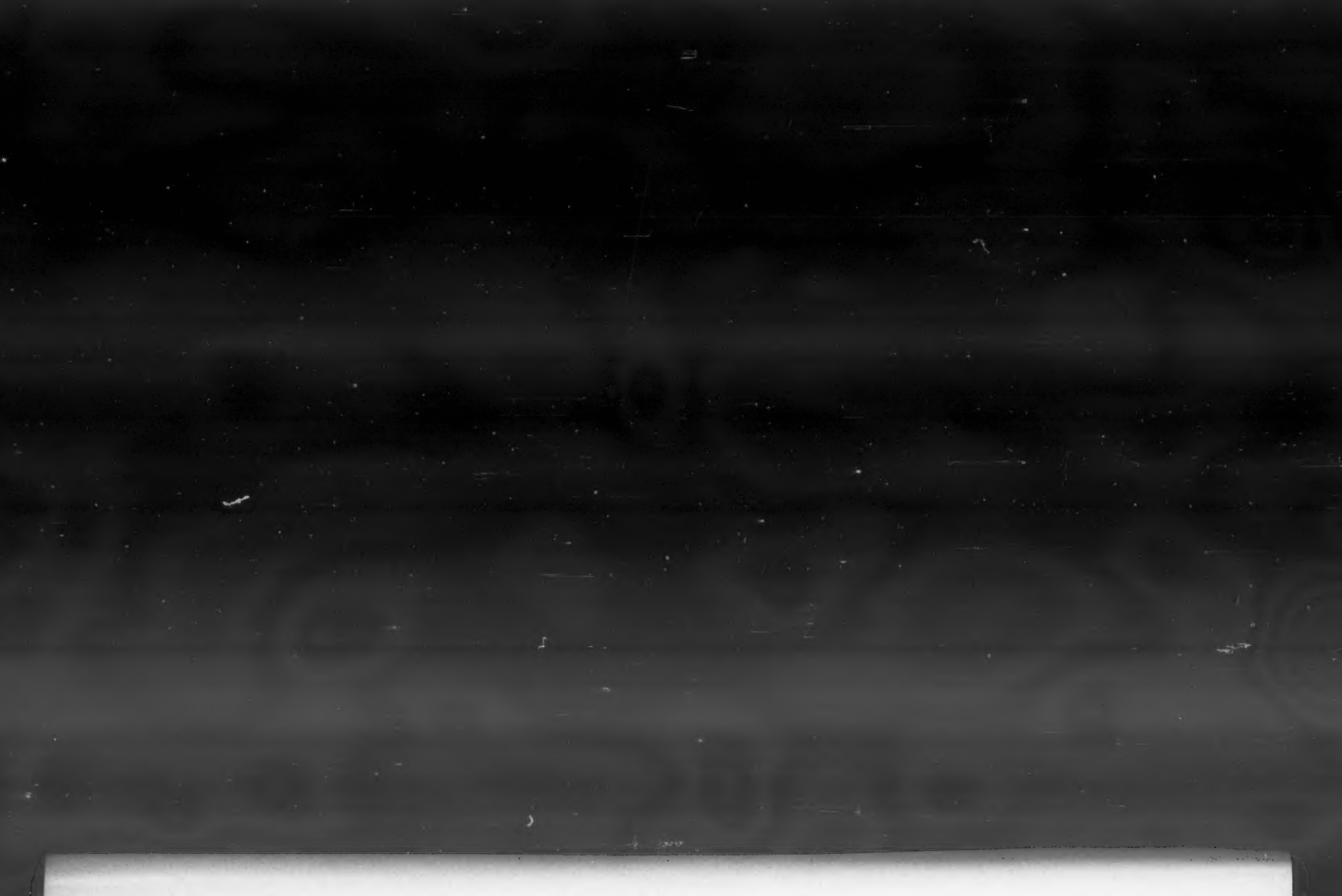

VESSEL
1
VESSEL
2
3

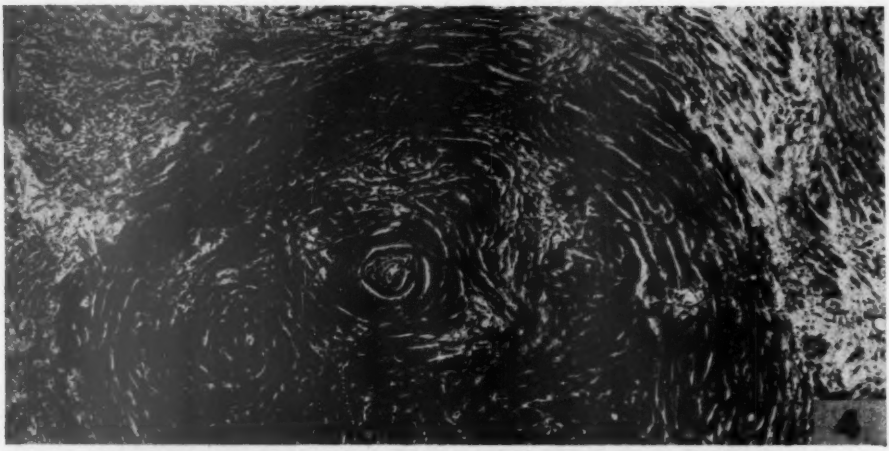
## PLATE 6

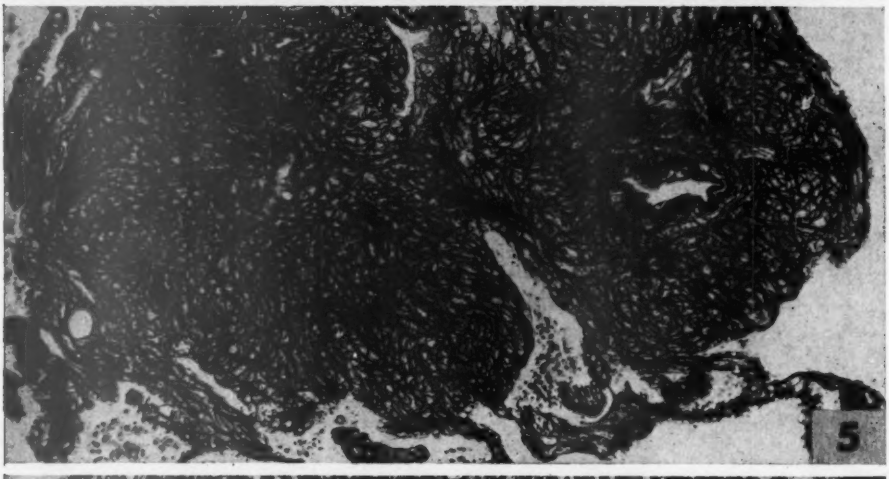
FIG. 4. Stationary silicotic nodule composed exclusively of thick collagen bundles. The limiting pneumonitis has become organized and the lymphatic spaces are not patent. × 70.

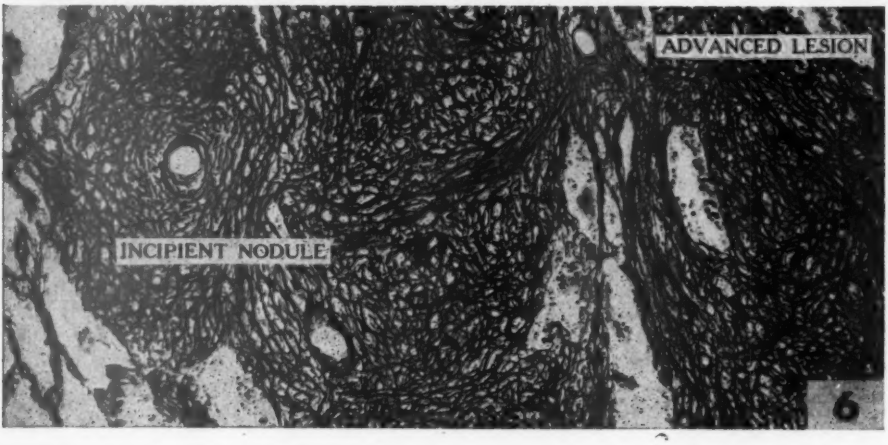
FIG. 5. Acinous silicotic nodule produced by the coalescence of two primary nodes, each one of them integrated by smaller secondary ones. It is difficult to define their limits because of the promiscuous interlacing of the reticular fibers. × 110.

FIG. 6. Silicotic nodule growing by "apposition." Incipient nodules appear near the edges of a more advanced lesion. The limit between the new lesion and the old is marked by a few collapsed alveoli. × 110.





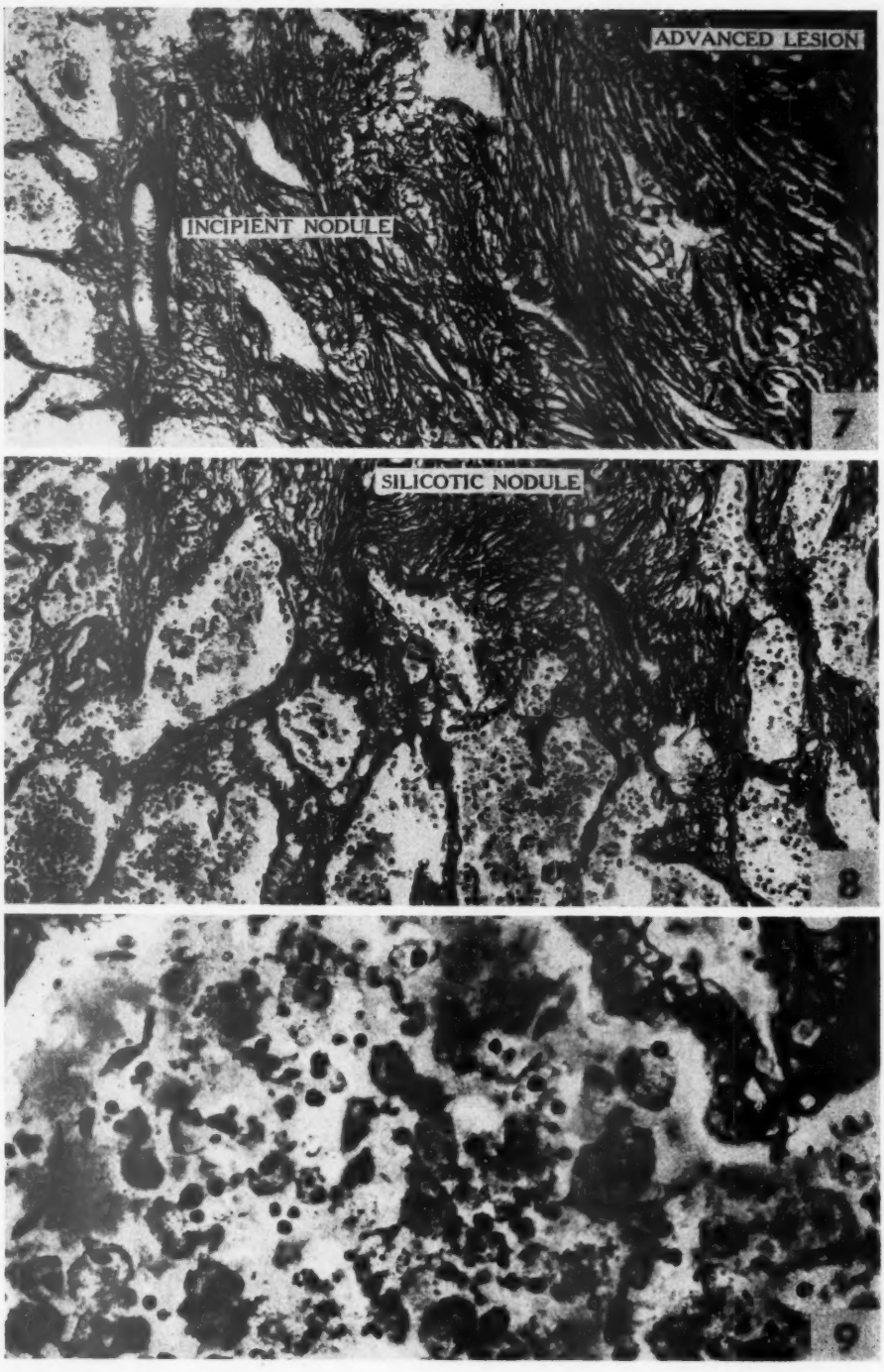

Costero Evolution of Silicotic Lesions

PLATE 7

FIG. 7. Edge of an advanced silicotic nodule growing by "apposition." The limit between the new lesion and the older one is less distinct than in Figure 6. × 110.

FIG. 8. Silicotic nodule growing by organization of desquamative pneumonitis. The alveoli contain desquamated cells and a small amount of albuminous fluid. × 110.

FIG. 9. Detailed view of the desquamative pneumonitis around a silicotic nodule in evolution. Alveolar macrophages are seen, some of them in necrobiosis, the majority in activity and with pseudopodial prolongations. × 440.



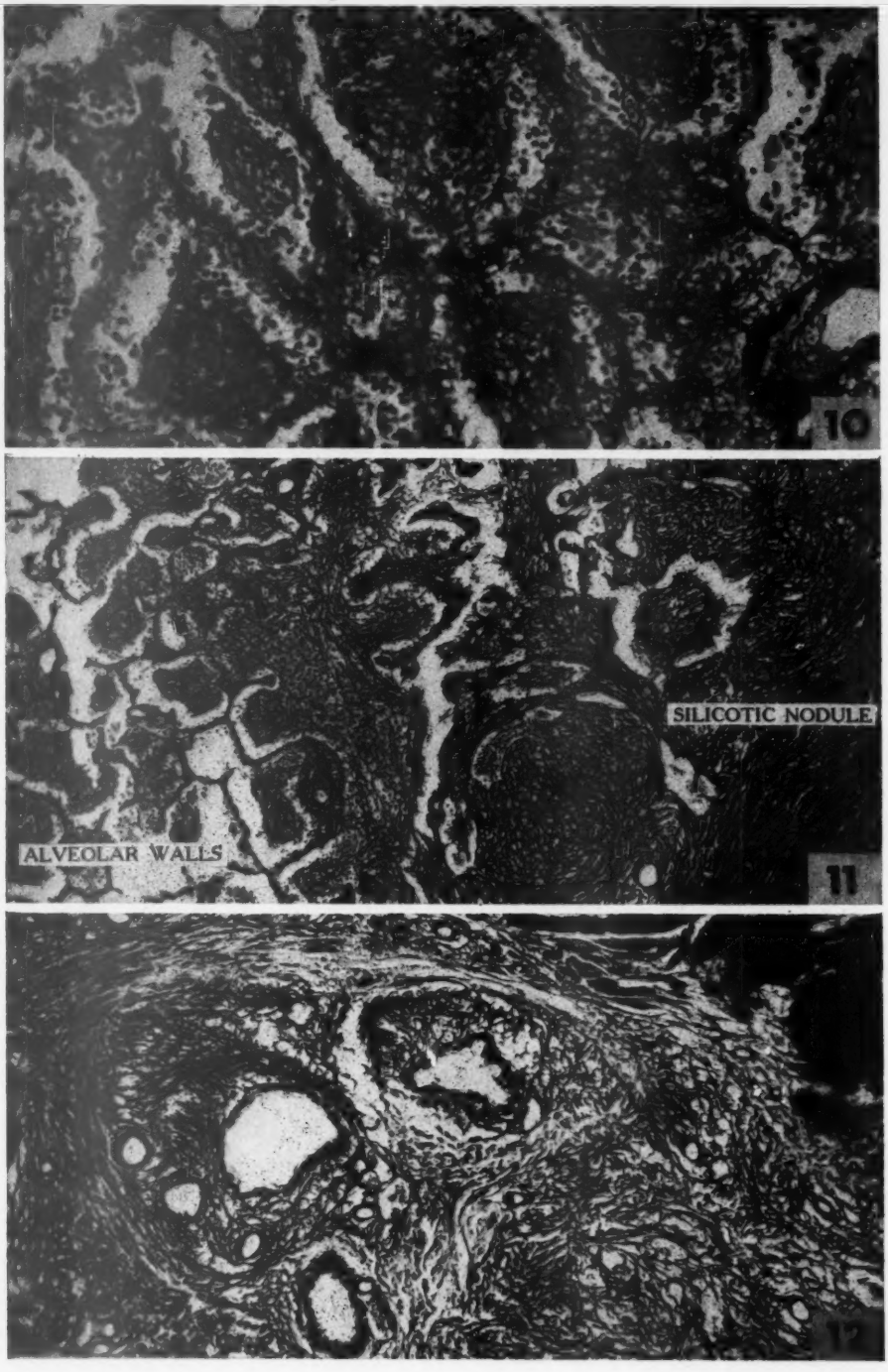

Costero Evolution of Silicotic Lesions

PLATE 8

FIG. 10. Organization of desquamative pneumonitis. The intra-alveolar fluid is being replaced by delicate reticular fibers, in part growing out from the reticulum of the alveolar walls and in part produced by desquamated histocytes. × 220.

FIG. 11. Limiting pneumonitis already organized and in coalescence with the edge of an older silicotic nodule. The partial conservation of the alveolar walls makes it possible to distinguish the pneumonic area. × 70.

FIG. 12. Growth of a silicotic nodule by transformation of atelectatic zones. In the right upper corner there is the border of an old collagen nodule; the remainder of the field is occupied by the atelectatic area. × 110.


10
SILICOTIC NODULE
ALVEOLAR WALLS
11
12

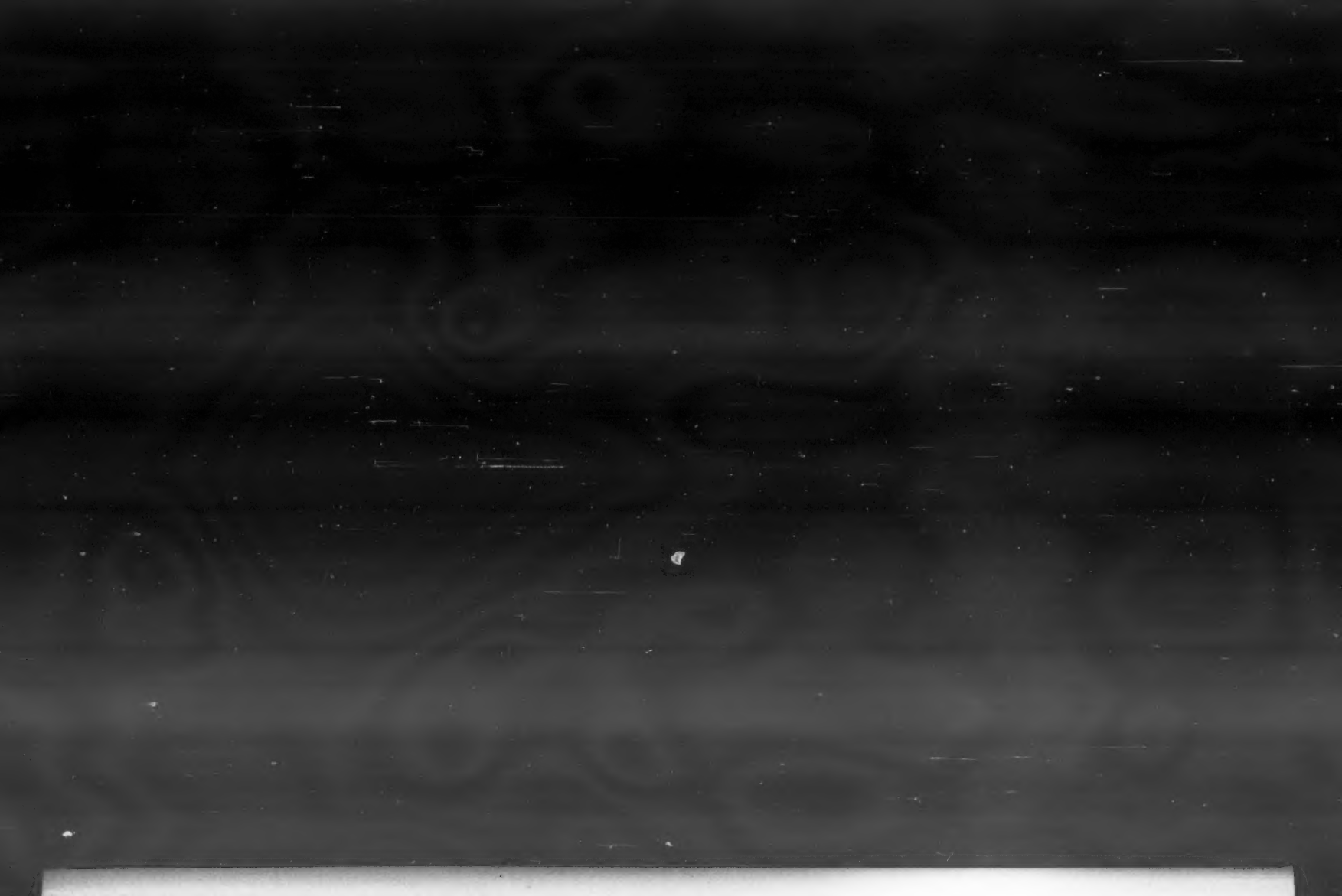
## PLATE 9

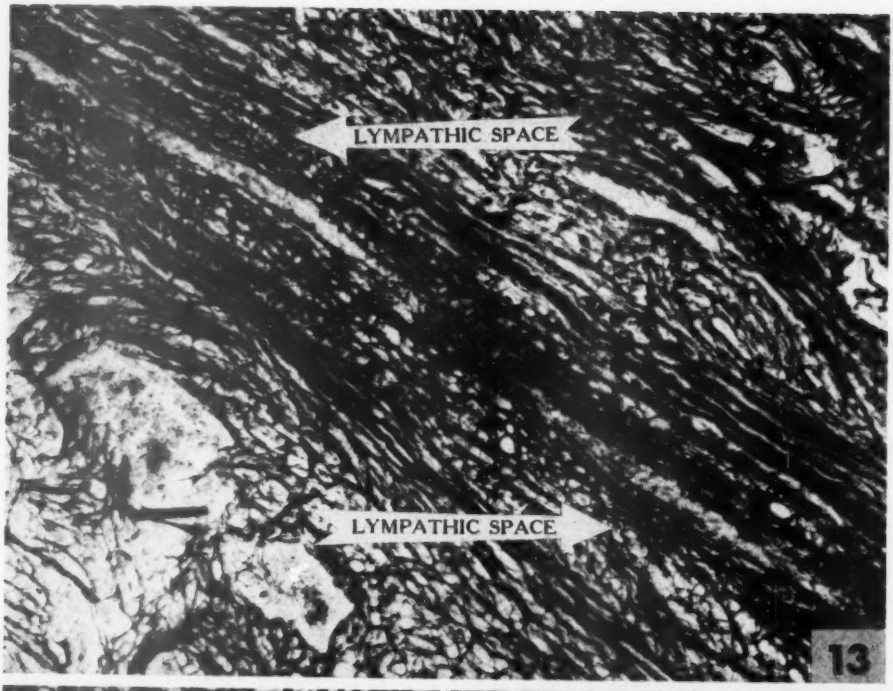
For "lympathic" read "lymphatic."

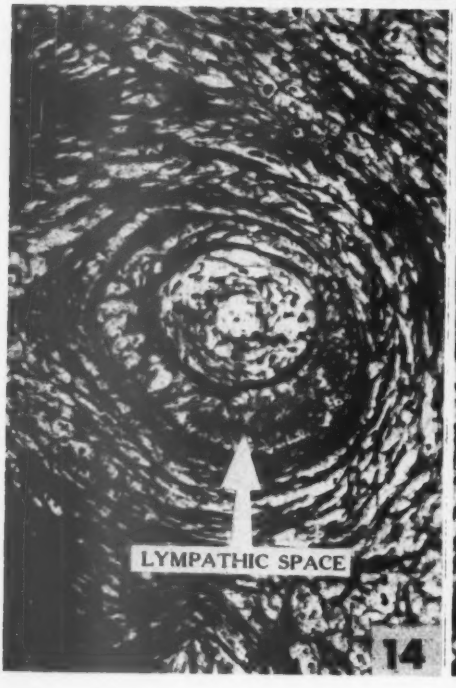
FIG. 13. Dilatation of the adventitial space of a blood vessel cut longitudinally within a silicotic nodule. The reticular fibers of the lymphatic space have proliferated. × 220.

FIG. 14. Another vessel similar to the one shown in Figure 13, but cut transversely. In the adventitial lymphatic space, which is dilated and almost obstructed by reticular proliferation, there are a few lymphocytes, macrophages, and siliceous particles. × 220.

FIG. 15. Central vessel of a silicotic node with more advanced changes than those in the preceding figures. The intima has proliferated and the lumen is completely obstructed. × 220.




LYMPATHIC SPACE
LYMPATHIC SPACE
13


LYMPATHIC SPACE
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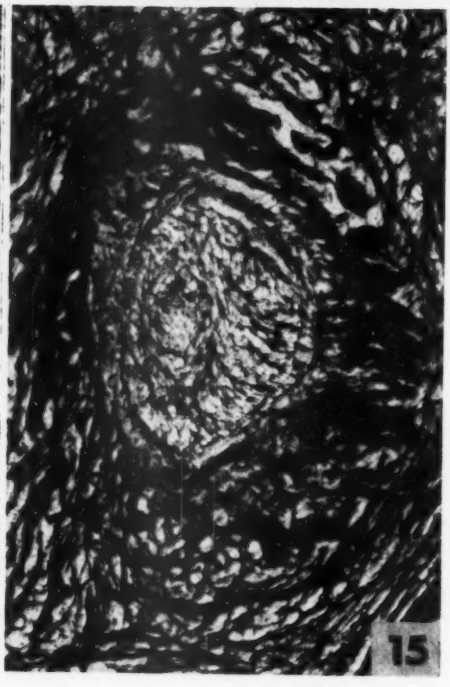
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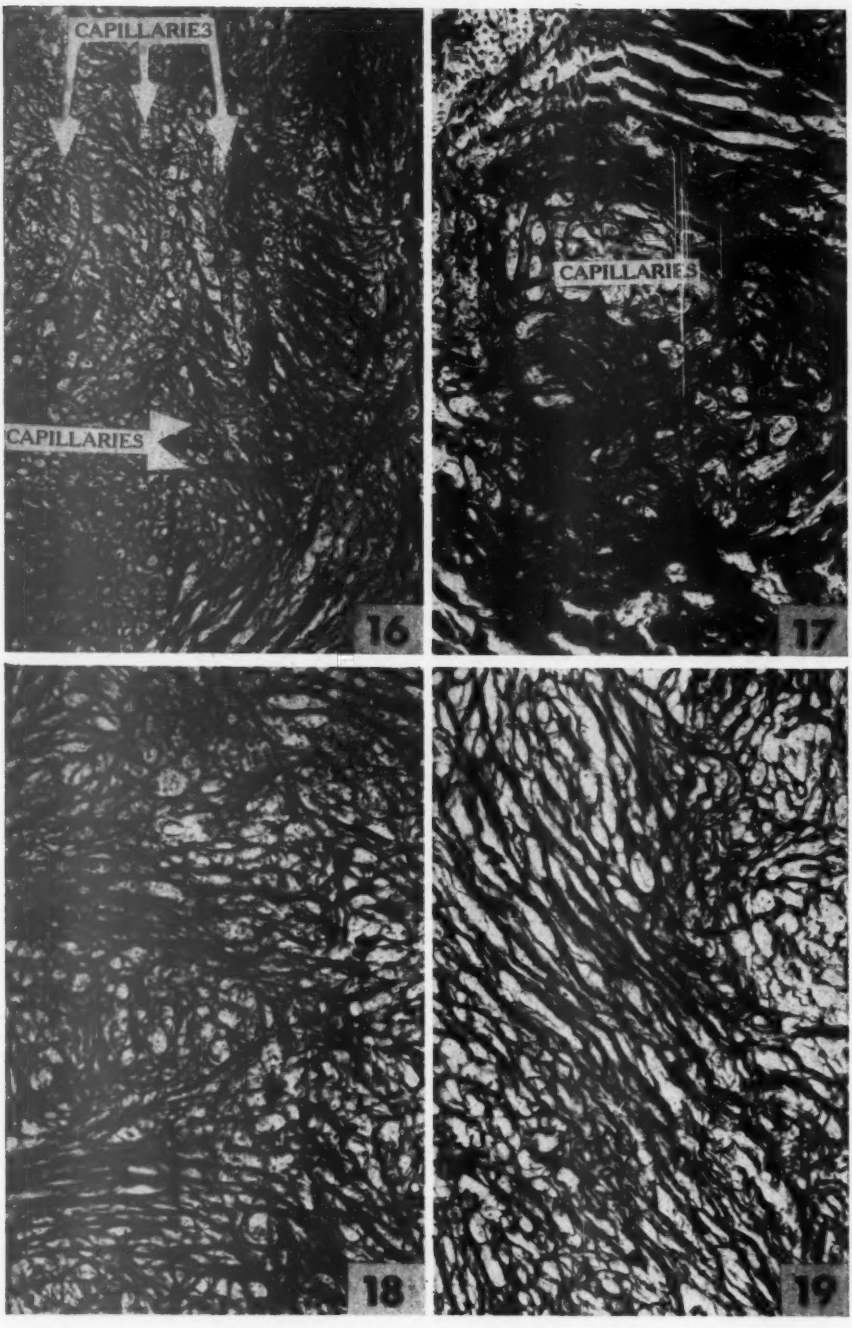
PLATE 10

FIG. 16. New formation of capillaries in a silicotic node in evolution. The vessels are clearly seen among the meshes of the reticular fibers and are similar to those observed in organizing thrombi and in foreign body reactions. × 110.

FIG. 17. Silicotic nodule during the process of collagen transformation with great numbers of newly formed capillaries arranged in a dense net of multiple anastomoses. Vascular proliferation contributes to the conservation of the reticular fibers. × 110.

FIG. 18. Persistence of the characteristic reticular structure of the argyrophilic fibers within a large silicotic nodule. Only a few filaments show a fascicular disposition. × 220.

FIG. 19. In this silicotic nodule the reticular structure of the argyrophilic fibers is partially lost. The fibers form parallel bundles without anastomoses, as is characteristic for collagen fibers. × 220.




Costero Evolution of Silicotic Lesions

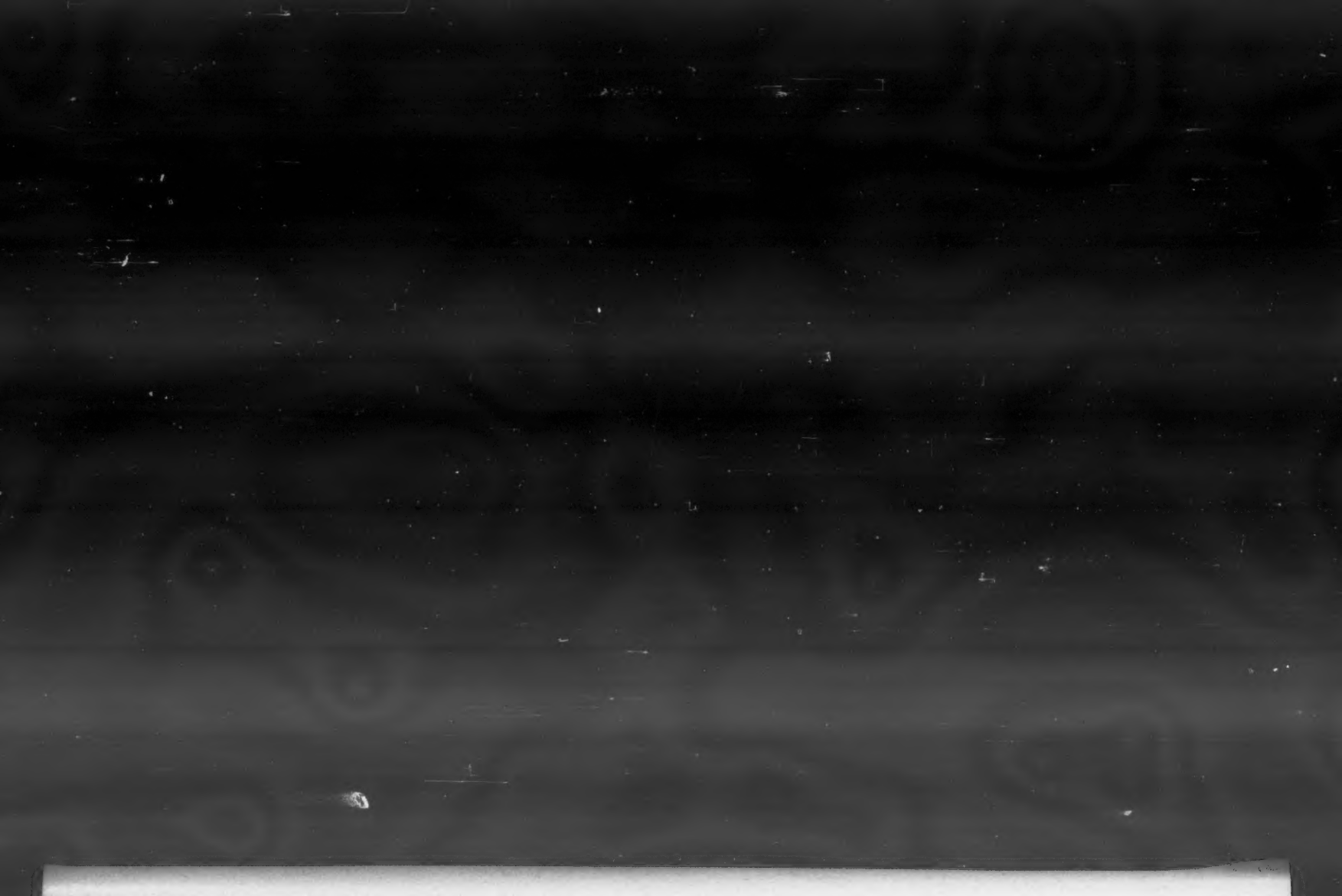
PLATE 11

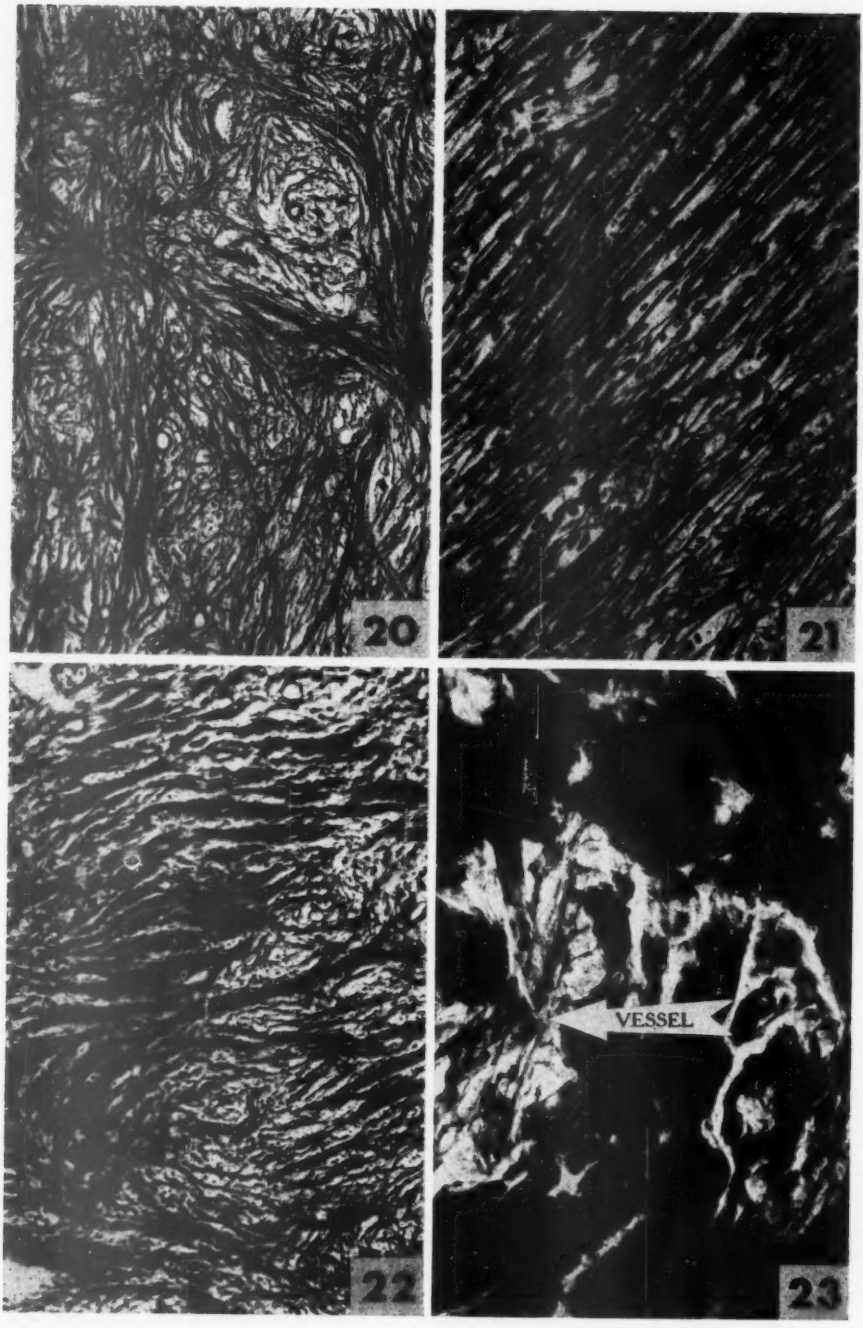
FIG. 20. Connective structure of a silicotic nodule at the beginning of collagenous transformation. There are several bundles of fibers but between them the picture is still predominantly reticular. × 110.

FIG. 21. Straight reticular fibers due to traction as a consequence of the shrinkage of the collagen fibers in neighboring silicotic nodules. × 220.

FIG. 22. Appearance of the first collagen bundles formed from the reticular fibers in the center of a silicotic nodule. The collagen fibers can be distinguished by their greater thickness, rectilinear arrangement, and lack of anastomosis. × 110.

FIG. 23. Center of a softening silicotic nodule at high magnification. In the loose area there are the débris of destroyed collagen fibers and a newly formed blood vessel. × 220.






Costero

**Evolution of Silicotic Lesions**

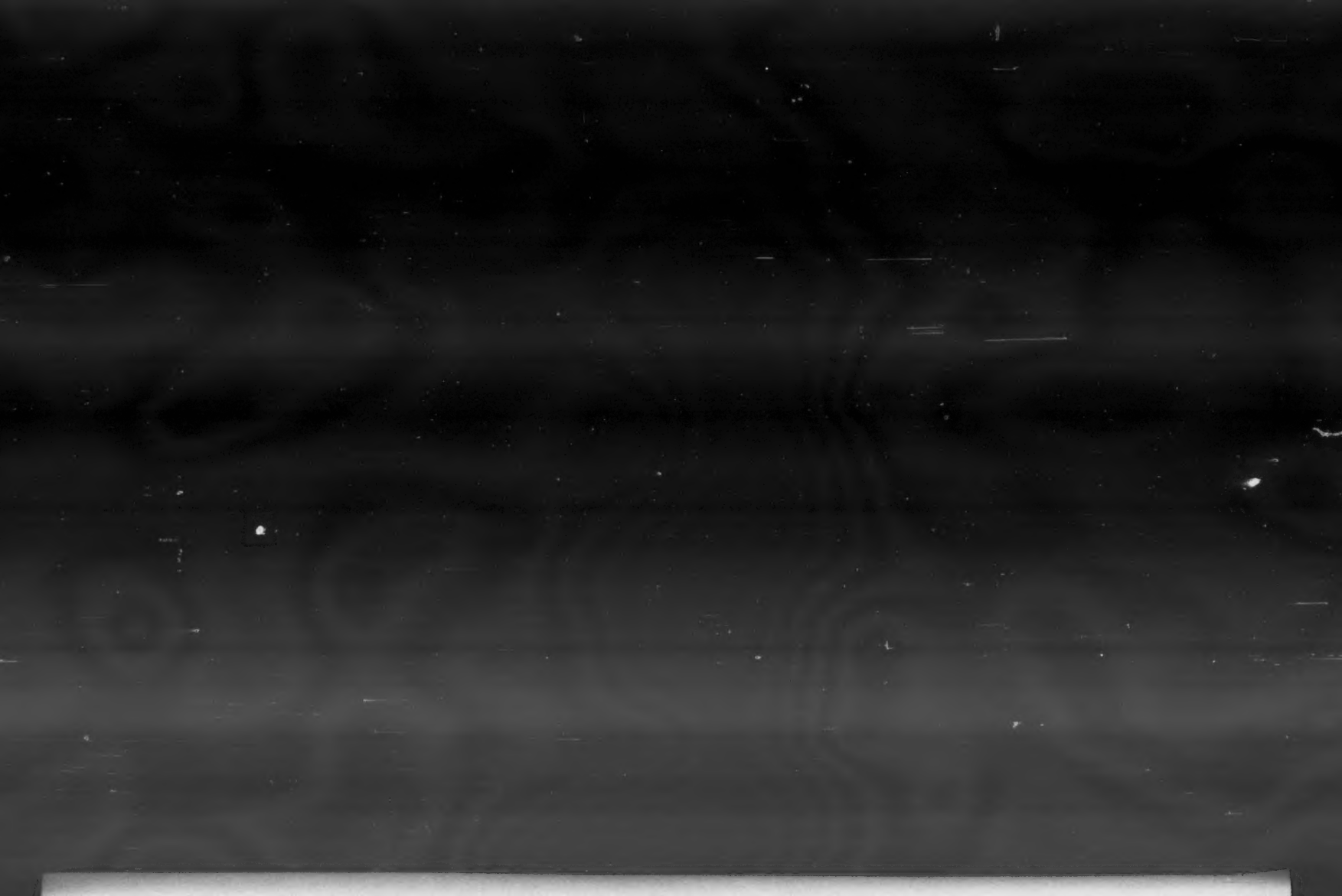
## PLATE 12

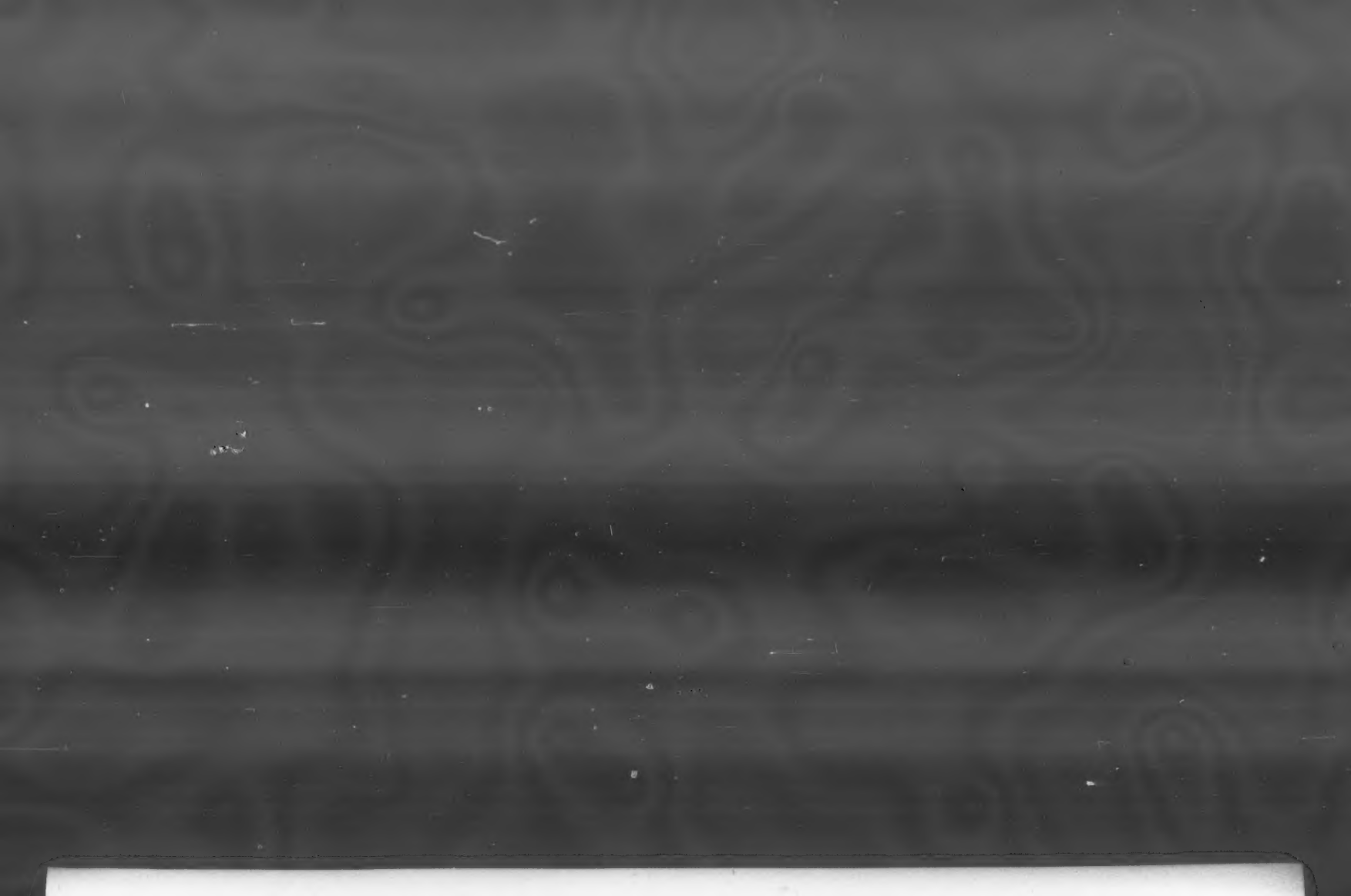
FIG. 24. Small silicotic nodule with its softening center occupied by lymphocytes. The canalizing vessel penetrates from the lower part of the field. × 110.

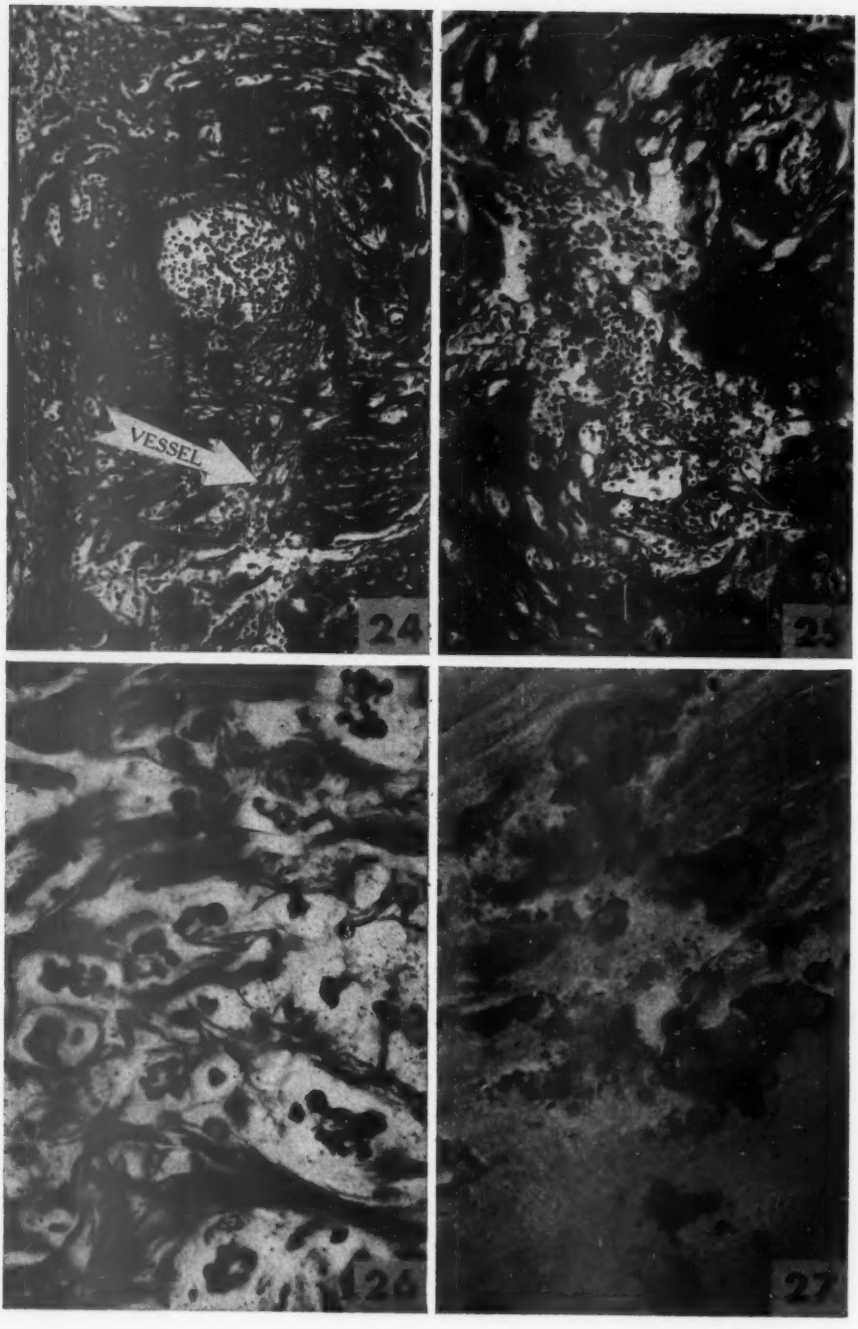
FIG. 25. To the vessels and lymphocytes in the softened center of the nodule, large quantities of active macrophagic cells have been added and their proteolytic enzymes have begun to destroy the collagen. × 110.

FIG. 26. Irregular chrysophilic masses with rounded edges, of unknown origin and significance, which are found between the fibers in the softened areas of some silicotic nodules. × 440.

FIG. 27. Large crystalline masses with indefinite edges, which sometimes appear in the softened areas of advanced silicotic lesions. × 680.








Costero Evolution of Silicotic Lesions

## Plate 13

Fig. 28. Macrophages arranged around the lymphatic vessels. These macrophages have their origin mainly from the histocytes of the alveolar walls. They are the carriers of the siliceous particles. × 110.

Fig. 29. Dense accumulation of macrophages as seen at a higher magnification than in Figure 28, showing the cytoplasm filled with siliceous particles. The accumulation of these cells is explained by the obstruction of lymphatic drainage. × 220.

Fig. 30. Detailed structure of the macrophages contained in silicotic nodules. The cytoplasm is highly developed and has a rounded shape. The phagocytized particles almost hide the nucleus. × 440.

Fig. 31. Macrophages caught between the hyalinizing collagen bundles; the retraction of these bundles contributes to the compression of the cells placed between them and thus to the atrophy of all living elements of the silicotic nodule. × 220.

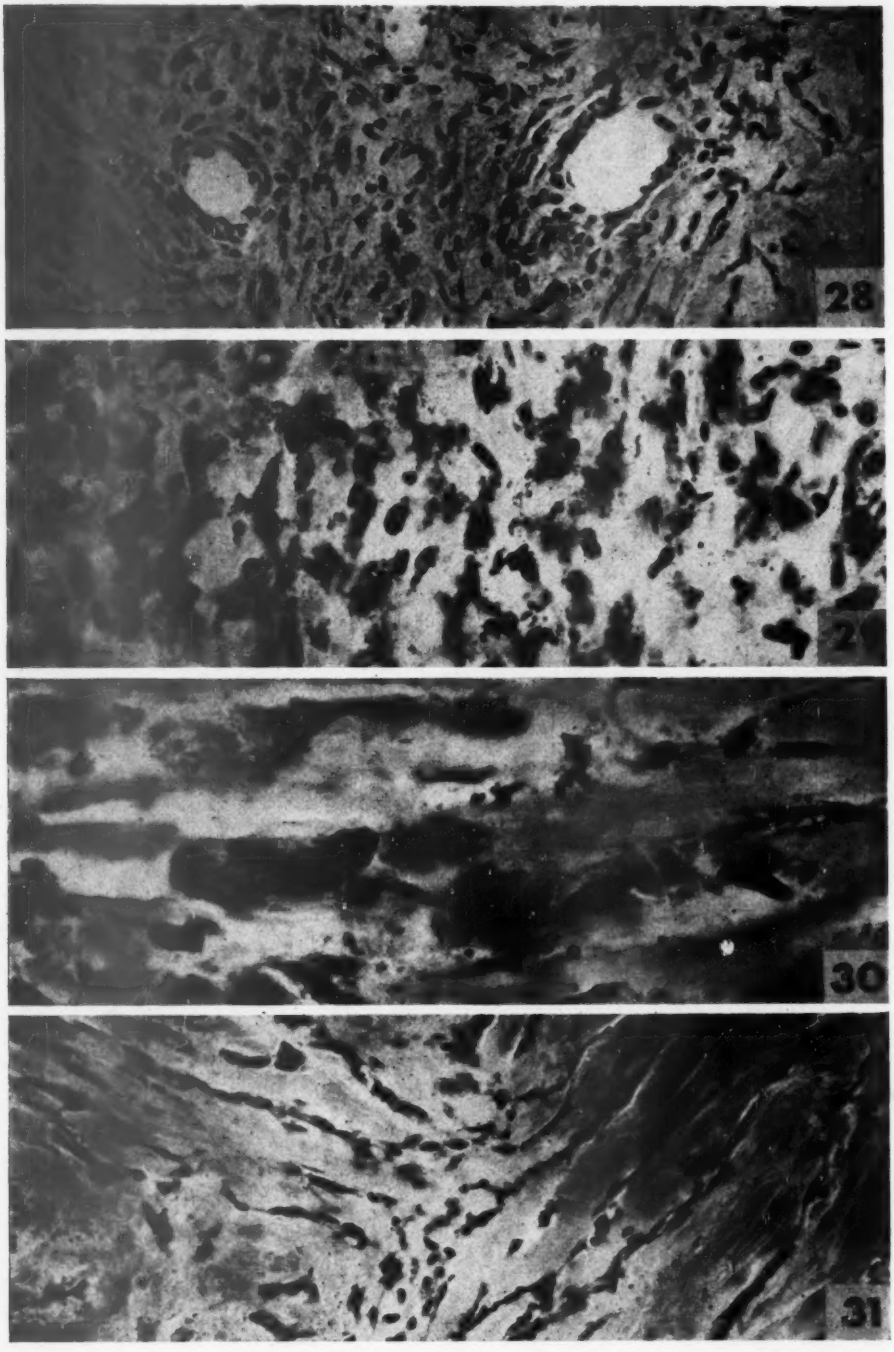

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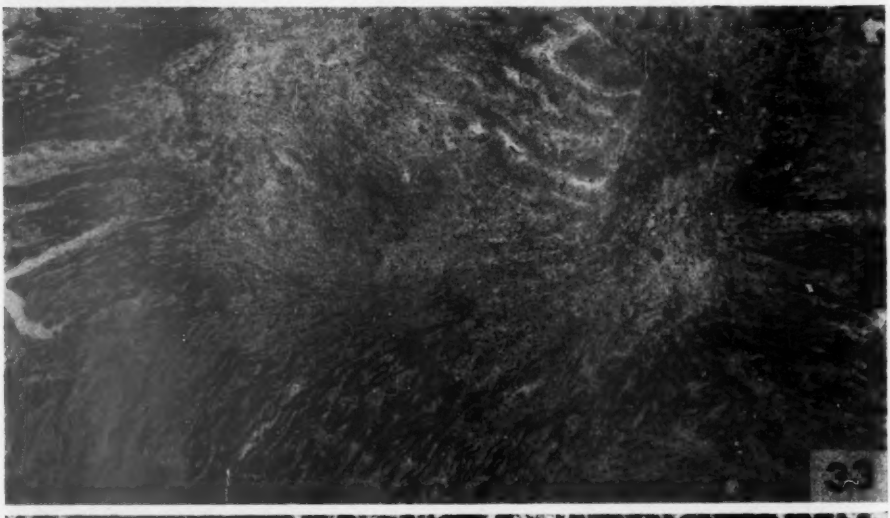
PLATE 14

FIG. 32. Macrophages in necrobiosis, with pyknotic nuclei and liquefying cytoplasm, within the softened area of an old silicotic nodule. The destruction of the cells in this case is due to autolysis, not to compression. × 220.

FIG. 33. Pulmonary tissue which contains a silicotic nodule, stained for elastic fibers. The fibrous part is lacking in elastic fibers, and in the neighboring alveolar walls the elastic fibers are distorted by the retraction of the sclerotic lesion. × 70.

FIG. 34. Diffuse sclerotic plaque representing a silicotic nodule deeply altered by vascular proliferation, multiple softening processes, and inflammatory phenomena. × 110.

32


33

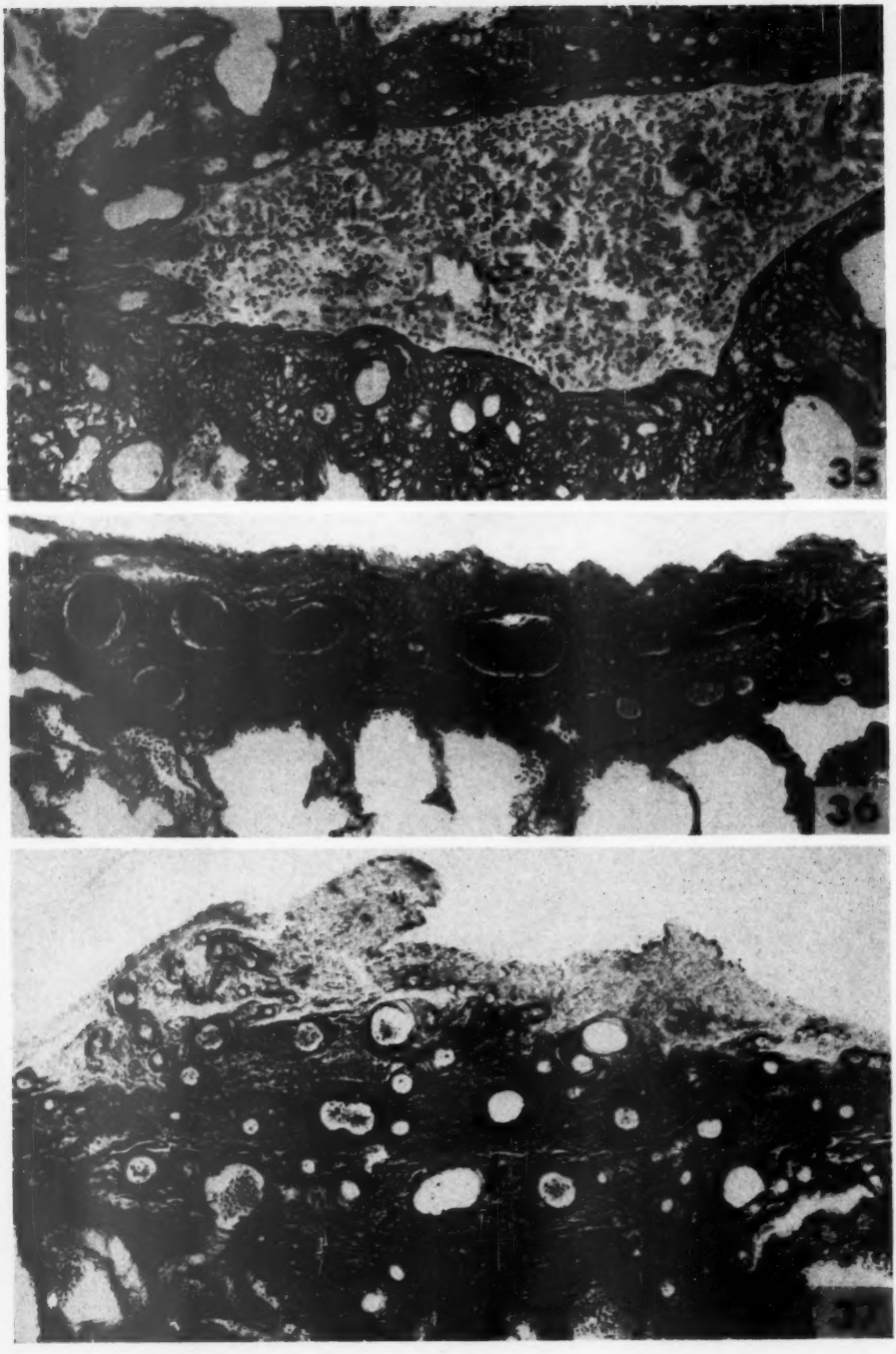
34

## Plate 15

Fig. 35. Peribronchial sclerosis. This lesion is due to factors similar to those which produce the typical silicotic nodule, modified by the special disposition of the lymphatic vessels along the bronchial tree. × 110.

Fig. 36. Thickening of the visceral pleura; blood and lymphatic vessels considerably dilated; hypertrophy of the reticular structure. These lesions show the first stage of silicotic pleuritis. × 70.

Fig. 37. Pleural silicotic plaque completely constituted. The newly formed capillaries are spread over the surface of the serosa and are accompanied by a very delicate precollagen network. × 70.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37

# TRUE ANEURYSMS OF THE MITRAL VALVE IN SUBACUTE BACTERIAL ENDOCARDITIS *

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Mycotic aneurysms of the aortic or mitral valve are not rare in patients with subacute bacterial endocarditis. These aneurysms are usually erosive in character in that they develop from an ulcerative endocarditis. Most commonly they consist of a hollow thrombotic mass attached to the valve. An erosion or ulcer of the involved cusp or leaflet leads into this cavity. To name such aneurysms correctly Ribbert[1] coined the term "thrombo-aneurysma." It is obvious that these are false aneurysms brought about by a destructive lesion in the valve. From the description in the literature of aneurysms found in association with subacute bacterial endocarditis, it is clear that almost invariably they are examples of such false erosive mycotic aneurysms. As early as 1867 Pelvet[2] was able to collect 9 examples of such aneurysms.

Von Arx[3] reported 4 instances of aneurysms of the heart valves. He concluded that they were false aneurysms and denied the existence of true nontraumatic mycotic valvular aneurysms. Ribbert,[1] in 1924, also studied the various mycotic valvular aneurysms but left the question open as to the existence of true valvular mycotic aneurysms. Such aneurysms, Ribbert remarked, would be the result of partial destruction of the valve leaflet, leaving a subendocardial layer intact, with subsequent replacement of the necrotic portion of the valve by granulation tissue. This eventually would give way to intracardiac pressure and form the wall of a true aneurysm.

Kaufmann[4] (1929) differentiated two types of aneurysms of the heart valves. One type is explained by the destruction of a layer of the valvular endocardium by progressive ulceration. Thrombotic masses and possibly necrotic valvular fragments form a considerable part of the wall of such an aneurysm, being thrust into the sac by the blood pressure. In other instances he asserted that the aneurysmal sac expands slowly until it reaches the size of a walnut, and then may contain thrombi or fluid blood. These latter aneurysms are referred to as chronic aneurysms. His Figure 10 depicts such a chronic aneurysm on the aortic tip of the mitral valve. However, Kaufmann does not distinguish between true and false aneurysms.

Pichon and Bidou,[5] in 1932, in a very short case report of subacute bacterial endocarditis, mentioned an aneurysm which was situated on

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the aortic leaflet of the mitral valve, bulging towards the atrial cavity. The aneurysm was nodular and disclosed a small point of rupture which was covered by a thrombus. The opening of the aneurysm measured 5 mm. The cavity was filled with clotted blood and its wall was very thin. There is no microscopic description, and there are no clear-cut illustrations. From the description it is not evident whether this was a true or false aneurysm. Soulié and Porge,[6] in 1937, also described an aneurysm of the aortic leaflet of the mitral valve in an instance of subacute bacterial endocarditis. The aneurysm measured about 3 cm. in greatest diameter, was saccular, and bulged into the left atrium. Its atrial surface was smooth and almost completely covered by endocardium. At the dome of the aneurysm was a small oval perforation which measured 5 mm. in greatest dimension. The aortic valve was retracted and fibrosed. Small vegetations and ulcerations were present on the aortic valve and adjacent to the aneurysm. Microscopic examination of the aneurysm disclosed destruction of the fibro-elastic tissue of the leaflet. It was replaced by inflammatory tissue which was partially necrotic. While from the gross description and the accompanying photographs it is evident that these authors described a true aneurysm, the microscopic description seems too inadequate to allow any definite conclusions as to its nature.

This study is based on the examination of 53 hearts with subacute bacterial endocarditis. Special attention was paid to the presence or absence of aneurysms of the valves. Such aneurysms were encountered in 12 cases. False aneurysms as described above were found in 7, and true aneurysms in 5 patients. All were located on the mitral valve. Only the true aneurysms form the basis of this study.

The diagnosis of subacute bacterial endocarditis was based on the evidence of an old valvular lesion, on the presence of relatively large vegetations, the involvement of the mural endocardium, and the discovery of *Streptococcus viridans* in the circulating blood of the patients at some time before their death. Also the prolonged clinical course of the disease was taken into consideration. The vegetations were rather firm, apparently partially organized, and occasionally areas of calcification were palpated. Though small ulcerations, particularly at the free margins of the involved valves, often were encountered in subacute bacterial endocarditis, they were not associated with true aneurysms. In 4 instances the older valvular lesions were obviously inflammatory, most likely of rheumatic origin. In one instance there was only an old syphilitic aortitis with involvement of the aortic valve. Vegetations, however, were present on both the aortic and mitral valves and the aneurysm was found on the anterior leaflet of the mitral valve.

In 4 instances the aneurysms were located on the aortic leaflet of the mitral valve and in one on the posterior leaflet. In one instance multiple aneurysms were encountered.

The morphologic appearance of these aneurysms was more or less similar. Each presented a well circumscribed, round, smooth outpouching or ballooning of part of a leaflet of the mitral valve. They measured from about 0.8 to 1.5 cm. in diameter. At first glance they resembled well organized, broad-based vegetations covered by smooth endocardium. Only by gentle compression of the sac-like structure was it possible to recognize the outpouchings as aneurysms. At the point of the highest convexity a small perforation was noted in 4 aneurysms. In only one a small, thin, fibrin thrombus was noted at the point of perforation. In 4 hearts small, firm vegetations were encountered at the periphery, adjacent to the bases of these aneurysms. These vegetations were whitish yellow and were covered by endocardium. In the one heart with several aneurysms they appeared in the form of soft, broad, oval folds with smooth surfaces covered completely by endocardium.

The ventricular surface of the aortic leaflet of the mitral valve was thickened and somewhat retracted in all instances, and, in 4, organizing and organized vegetations covered by endocardium were found. On the ventricular surface of this leaflet, corresponding to the aneurysms which bulged towards the atrium, were small, round defects with smooth but thickened margins. These defects formed the openings into the aneurysms. The defects or entrances into the aneurysmal cavities were much smaller than the aneurysms themselves. When the aneurysms were incised, cavities were encountered containing blood or small thrombi which were only loosely attached to the wall of the aneurysm.

Portions of the aneurysms were examined histologically. A number of sections were cut from the involved leaflet and aneurysmal wall. The sections were stained with hematoxylin and eosin, iron hematoxylin, orcein, and according to the van Gieson method. Also the Giemsa stain was used when deemed necessary.

In the microscopic description the nomenclature of the various layers of the mitral leaflets as used by Gross and Kugel [7] will be adhered to. The ventricularis of the aortic leaflet of the mitral valve adjacent to the aneurysm disclosed thickened elastic lamellae. In the region of the aneurysm, however, the lamellae were thinned out, and splitting of the elastic fibers was noted. Between them was a cellular connective tissue with a few lymphocytes and endothelial leukocytes. The fibrosa which normally forms the predominate part of the leaflet was severely reduced. The parallel arrangement of the collagenous fibers could not

be made out. Certain fibers were much thicker than is normal while others were replaced by young connective tissue. A moderate number of lymphocytes and endothelial cells were present.

A few newly formed blood vessels were encountered, some of which were dilated to form small blood sinuses. No distinct spongiosa was recognized, the thinned-out fibrosa being covered directly by the auricularis. Whereas normally, as Gross and Kugel[7] stated, a continuation of the endocardial smooth muscle can usually be traced down the auricularis layer, covered by auricularis elastic lamellae, no evidence of smooth muscle was present in the region of the aneurysm. The elastic fibers were broken, thinned out, poorly stained, and often split into thin fragments which formed irregularly outlined masses of elastic tissue. Inflammatory cells were more numerous in this layer than in other portions of the wall of the aneurysm. A few polymorphonuclear leukocytes in addition to endothelial leukocytes and lymphocytes were encountered. The endothelial layer over the aneurysm was intact. However, the normally present jelly-like zone between the endothelial layer and most superficial elastic fibers could not be seen. At the very tip of the convexity of the aneurysms only a small amount of thin collagenous tissue was seen, covered by endothelium.

Microscopic sections taken from various other portions of the valve disclosed granulation tissue and scar tissue. Organized and organizing vegetations were present. Often granulation tissue was noted at the base of the vegetations while their more distal parts consisted of fibrin with polymorphonuclear leukocytes and much bluish-staining amorphous material. The latter contained no bacteria but gave the staining reaction of calcium salts as was described in a previous study.[8] Some of the vegetations were completely organized and covered with endothelium.

To summarize the foregoing: In 5 instances of subacute bacterial endocarditis true aneurysms were found on the mitral valve. These aneurysms were definitely not "thrombo-aneurysms" (Ribbert[1]), or false aneurysms, but the result of valvulitis with consequent formation of granulation tissue and scar tissue which succumbed to intraventricular pressure with the formation of sac-like outpouchings. Four of the 5 aneurysms had a recent tear at the tip of their convexity.

As stated above, these 5 valvular aneurysms were encountered among 53 patients with subacute bacterial endocarditis. These aneurysms were encountered between 1943 and 1946. In none of 41 hearts with subacute bacterial endocarditis observed between 1935 and 1943 were true aneurysms found. False aneurysms (thrombo-aneurysms) were found 7 times in this period. The 5 true valvular aneurysms were noted

in 5 of 12 hearts with subacute bacterial endocarditis in the autopsy material from 1943 to 1946. In order to find factors which could explain the increased incidence of these aneurysms, the records of the patients presenting these lesions were reviewed and compared with those of previous years. No difference was noted in the early clinical picture of the disease and in the general pathologic changes in the two groups. The organism most frequently found in the blood was *Str. viridans*. However, in those instances in which valvular aneurysms were observed, the patients had received massive doses of penicillin associated sometimes with sulfonamide drugs and heparin. The clinical course of these patients was more protracted and 2 had been pronounced cured. Previous to 1943 the treatment was mainly palliative and symptomatic, and such chemotherapy as was used had been inadequate.

From the histologic examination of the aneurysms it seems clear that the healing process of the valvulitis formed a *locus minoris resistentiae*. The changes were found principally in the zona fibrosa of the valve which normally constitutes its largest part. This region, being replaced by granulation tissue and young scar tissue, succumbed to the intracardiac pressure and formed the aneurysms.

Paradoxical as it may seem, these aneurysms thus may be regarded as evidence of healing and indicate at least partially successful treatment of patients with subacute bacterial endocarditis. Libman and Friedberg,[9] in their monograph on subacute bacterial endocarditis, mentioned the presence of mycotic aneurysms not only in the arterial system but also on the heart valves. They did not classify these aneurysms into true or false aneurysms. However, from their gross description of the aneurysm of the aortic valve as "ballooning of the cusp" and from the accompanying picture it is clear that they found true aneurysms, although there are no microscopic descriptions of them. It is noteworthy that these authors found such aneurysms in the so-called bacteria-free stage of subacute bacterial endocarditis. It seems to us that "bacteria-free stage" implies some progress towards healing. These authors also mentioned an instance of "ballooning of the aortic cusp" in a patient who died 8 years after an attack of subacute bacterial endocarditis. Rosenblatt and Loewe [10] described, in an instance of healed subacute bacterial endocarditis, an aneurysm-like sac in the aorta just above the attached margin of one of the cusps of the aortic valve. The base of the right auricular appendage covered the roof of the aneurysm. Although the authors believed that this aneurysm constituted a congenital anomaly, from the foregoing it seems that this lesion might very well have been a true mycotic aneurysm as here described,

and thus evidence of healing or healed subacute bacterial endocarditis.

In 4 of the 5 patients in this series the aneurysms had ruptured. Since the aneurysms were located on the mitral valve, the rupture obviously caused an insufficiency of this valve or increased a pre-existing insufficiency. From the gross and histologic appearance of these ruptured areas it is obvious that the ruptures must have occurred shortly before death. It is likely that the suddenly increased burden upon the myocardium was a contributing and final factor in the death of these patients.

Modern treatment has altered the mortality rate of subacute bacterial endocarditis. This is due to the fact that the infectious agents of this disease are made innocuous. It has been shown that some of the patients treated successfully as far as the infection was concerned, died some time later because of the concomitant myocardial damage.[8] This study shows that some of these patients may die as the result of valvular aneurysms incident to the use of the newer therapeutic methods.

## Summary

True aneurysms of the mitral valve, mycotic in nature, have been noted 5 times among 12 patients with subacute bacterial endocarditis during the period between 1943 and 1946. These patients had been treated with various sulfonamide drugs, heparin, and penicillin. Among 41 patients with subacute bacterial endocarditis autopsied between 1935 and 1943 there was not a single instance of true aneurysm. Grossly, the aneurysms resembled broad-based, healed vegetations. Only on palpation was their nature recognized. These aneurysms are the result of a circumscribed valvulitis with consequent granulation tissue and young scars which, due to intracardiac pressure, formed sac-like outpouchings. All of these aneurysms were covered by the valvular endothelium. Four of them had ruptured shortly before the death of the patient. The sudden increase of incompetence of the mitral valve was a contributory factor in the death of these patients. These aneurysms are considered as evidence of healing of the valvular lesions in subacute bacterial endocarditis. Their increasing frequency may be attributed to the use of modern therapeutic agents.

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[ *Illustrations follow* ]

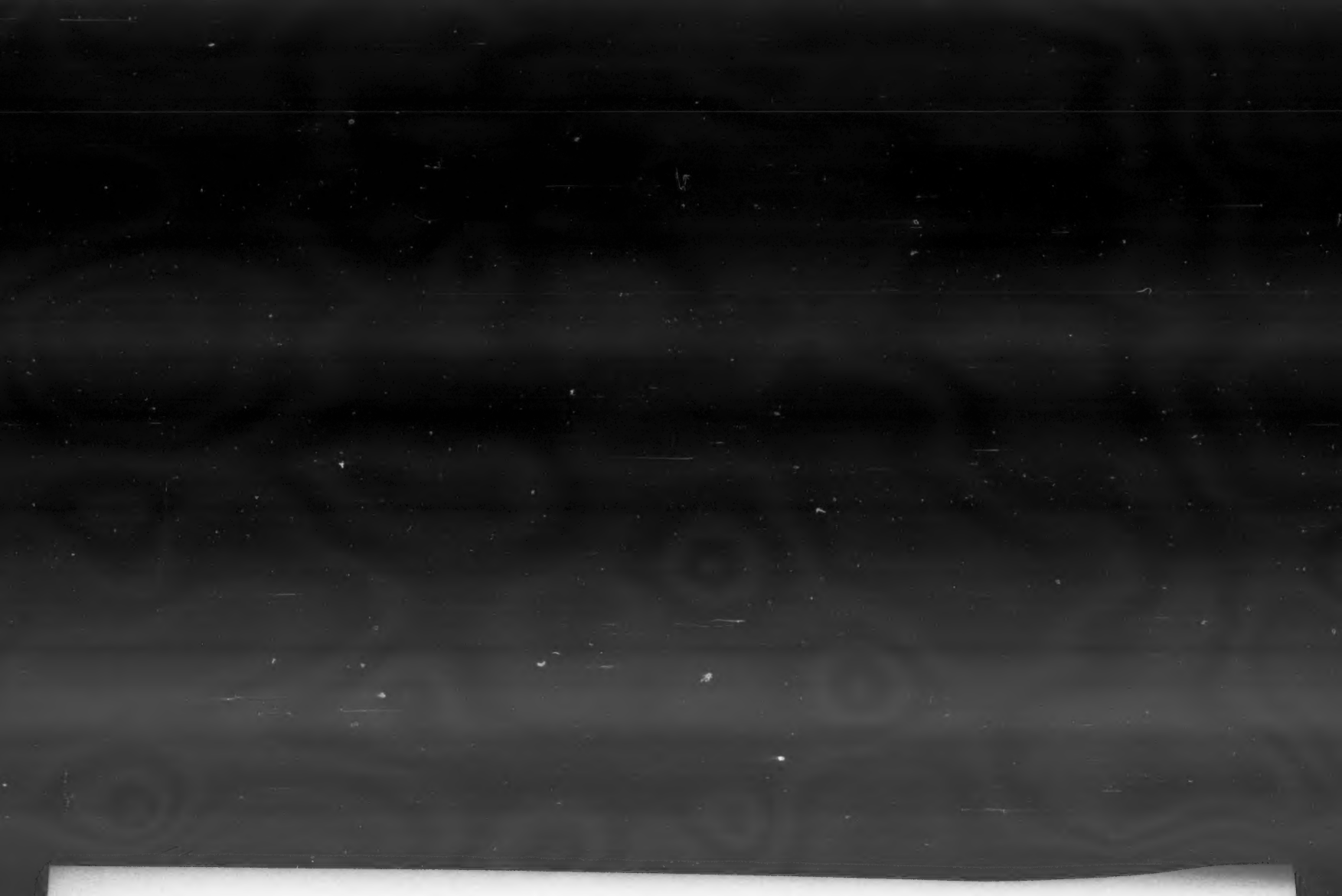
## DESCRIPTION OF PLATES

### PLATE 16

FIG. 1. Aneurysm of the mitral valve. The area of perforation disclosed a small thrombus.

FIG. 2. Aneurysm of the mitral valve. Healing vegetations surround the aneurysm.

FIG. 3. The aneurysm shown in Figure 2, moderately enlarged.



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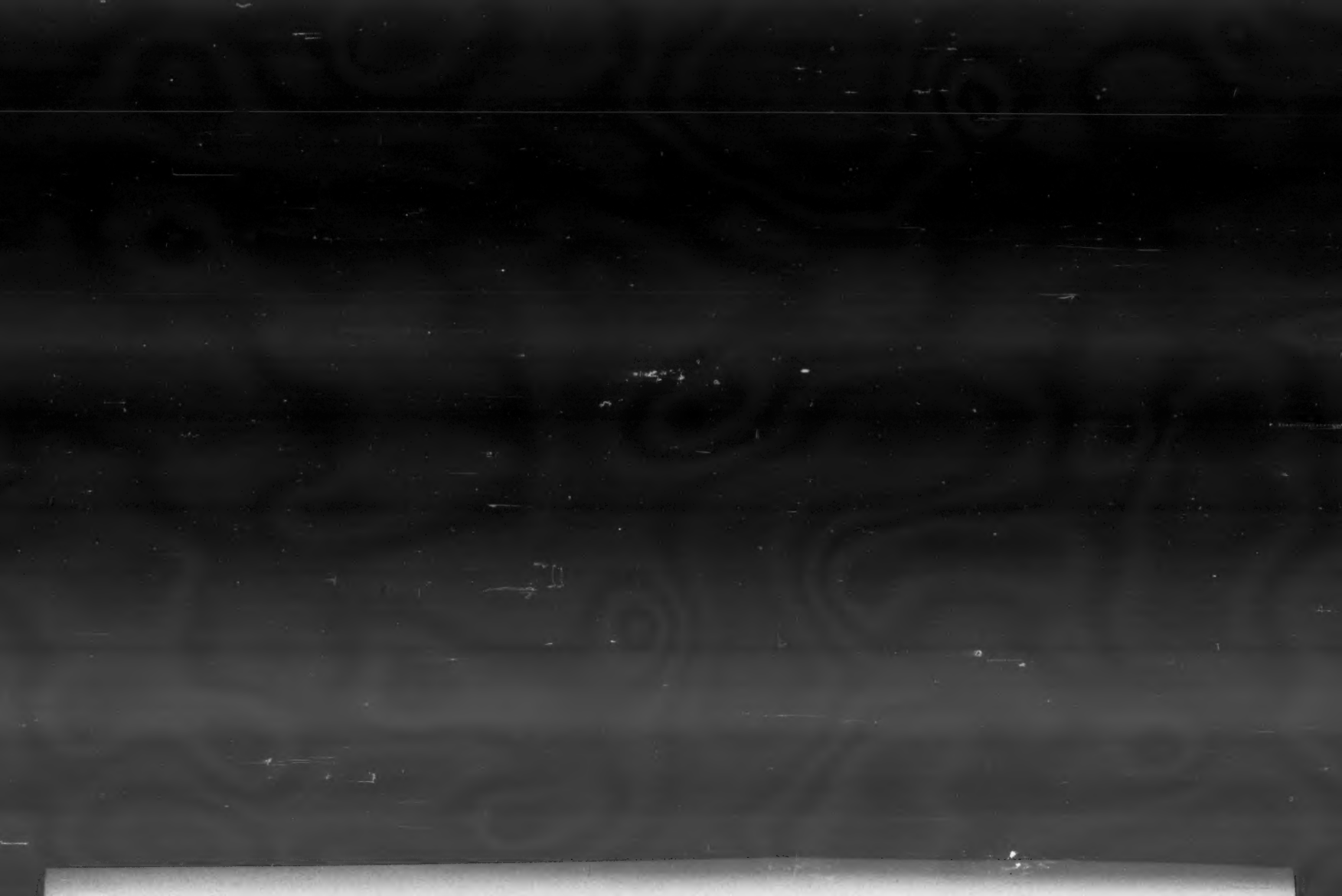
Saphir and Leroy Aneurysms of the Mitral Valve

PLATE 17

FIG. 4. Multiple aneurysms of the mitral valve.

FIG. 5. Ventricular aspect of one of the aneurysms (entrance into the aneurysm). Of note are the partially organized vegetations just above the opening into the aneurysm and on the aortic valve.

FIG. 6. Ventricular aspect of the aneurysm, showing the smooth margin.



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## Plate 18

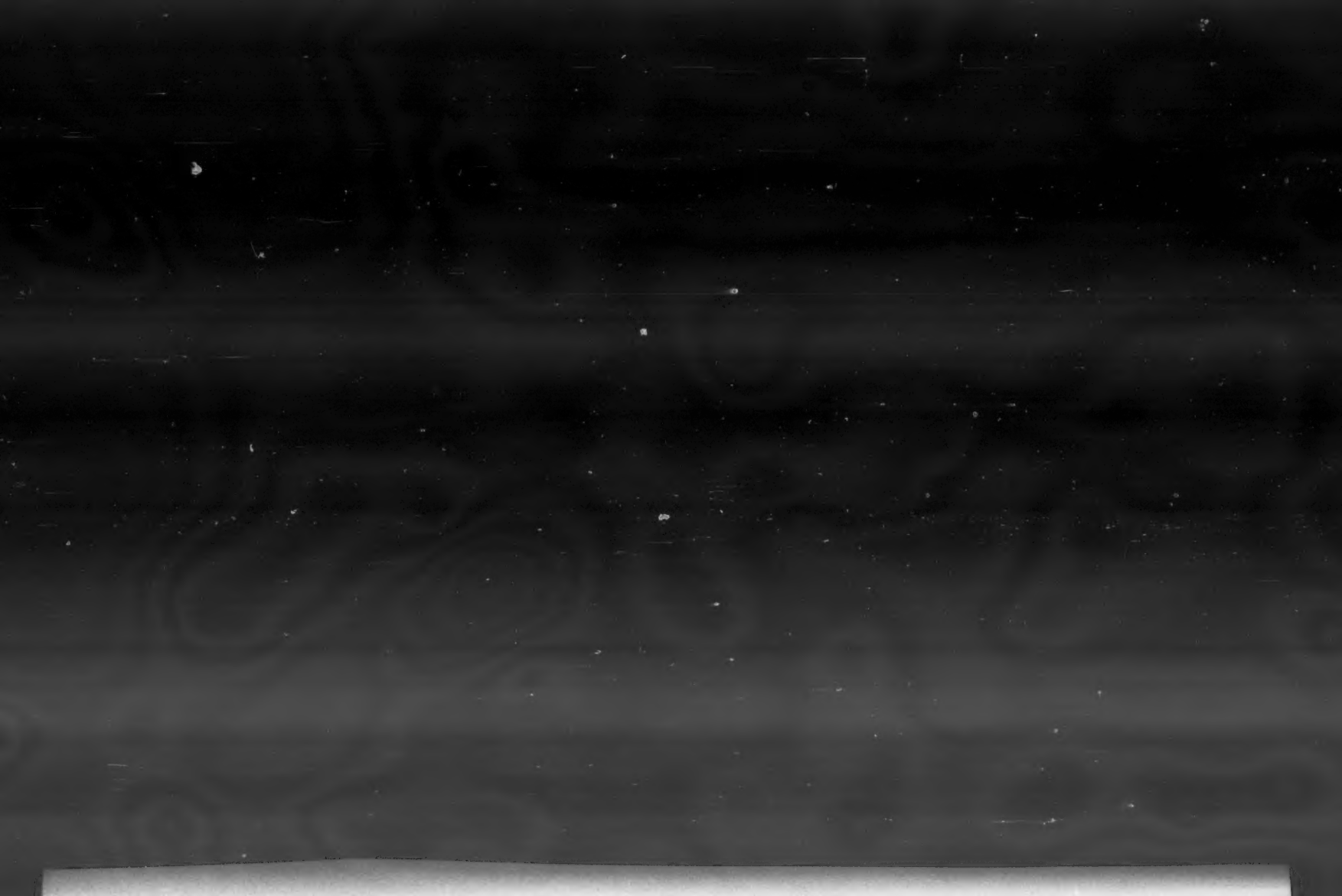
Fig. 7. Section of the mitral valve adjacent to the aneurysm. Of note are the small-sized blood vessels and inflammatory cells. Iron-hematoxylin and eosin stain. × 150.

Fig. 8. Mitral valve, region of the aneurysm. Granulation tissue is present. Iron-hematoxylin and eosin stain. × 300.

Fig. 9. Mitral valve, auricular aspect. Of note are the fibroblasts in the region of the aneurysm, and the normal covering by endothelium. Iron-hematoxylin and eosin stain. × 150.

Fig. 10. Blood sinuses are present in the region adjacent to the aneurysm. Iron-hematoxylin and eosin stain. × 150.

Fig. 11. Mitral valve, region of the aneurysm, showing splitting of the elastic lamellae and the intact zona auricularis. Iron-hematoxylin and eosin stain. × 150.



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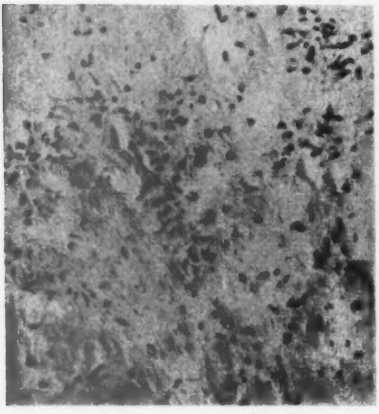

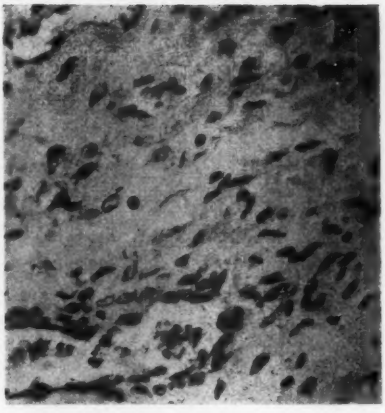

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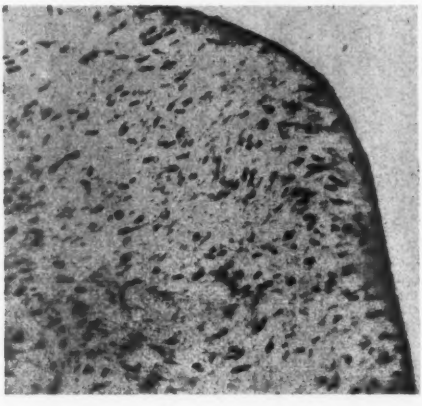

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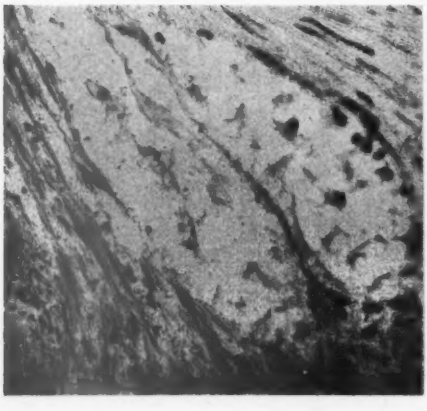

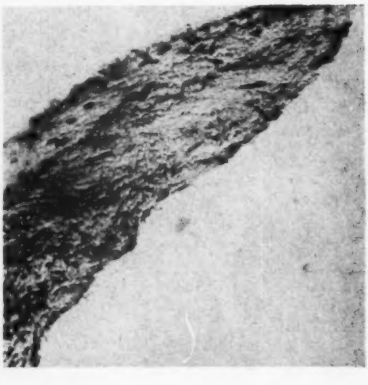

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Saphir and Leroy

Aneurysms of the Mitral Valve

# VIRUS MYOCARDITIS
## PATHOLOGIC AND EXPERIMENTAL STUDIES *

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In 1899 Fiedler [1] described isolated myocarditis in man, and a careful review of the literature since that time discloses many reports of this condition in both man and animals. Moreover, a long list of bacterial and other agents have been suggested as etiologic factors in many of these reports. The lesion has been recorded under a variety of names; for example, Fiedler's myocarditis, myocarditis of unknown etiology, allergic, isolated, primary, interstitial, circumscribed, diffuse, and idiopathic myocarditis. The microscopic findings in these recorded cases have stressed polymorphonuclear and mononuclear interstitial infiltration, often with necrosis of myocardial fibers.

Since Fiedler's original description,[1] myocarditis apparently has been produced under variable conditions in several different laboratory animals but without sufficient changes for gross identification. In 1924 Miller [2] described a myocardial lesion occurring in 60 per cent of 34 rabbits which had been known to be otherwise healthy for periods varying from 2 to 124 days. Microscopically, these lesions consisted of lymphocytes and endothelial leukocytes, and only occasionally were polymorphonuclear eosinophils, plasma cells, and fibroblasts seen. Spontaneous myocarditis with slight inflammatory changes was reported in about 30 per cent of one series of Swiss strain mice.[3] Myocarditis has been found in middle-aged mice, between 10 to 20 months of age, accompanied by pleuropericardial hemorrhage and frequently by testicular hemorrhage. Furthermore, inoculations of heart tissue from 7 mice with myocarditis into 74 other mice by various methods were without results, nor could a causative agent be isolated.[4] Torres [5] described spontaneous interstitial myocarditis in apparently healthy dogs. The cellular infiltrations were chiefly perivascular in distribution and were composed of lymphocytes, macrophages, and ameboid wandering cells. Other investigators have described myocarditis in animals occurring in cases of both mineral and protein deficiencies.[6,7]

In 1942, French and Weller [8] described interstitial myocarditis occurring in human beings, rabbits, rats, and mice following the use of neoprontosil, sulfanilamide, sodium sulfapyridine, and sodium sulfathiazole. The cellular infiltration of this lesion consisted of large mononuclear cells of clasmatocytic type and numerous cells with granular

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eosinophilic cytoplasm, a few of which were polymorphonuclear. Experimentally, they produced the disease in 38 of 60 mice and in 33 of 40 rats. Spontaneous myocarditis occurs occasionally in rabbits, and Pearce [9] was able to produce myocardial lesions consistently in rabbits by introducing a solution of acacia immediately prior to inoculation of vaccine virus, the myxoma virus, or two strains of the fibroma virus. He obtained cardiac lesions with necrosis and an inflammatory reaction which differed only slightly from virus to virus. In 1945 Finland and co-workers [10] isolated the influenza A virus from the lungs of 2 patients dying of interstitial myocarditis. The heart fibers showed focal necrosis and invasion by large mononuclear cells. The interstitial tissues were infiltrated with large mononuclear cells, lymphocytes, plasma cells, eosinophils, and polymorphonuclear leukocytes. In 1945 Helwig and Schmidt [11] isolated a virus from a group of anthropoid apes dying from interstitial myocarditis and produced myocardial lesions consistently in mice. This report is concerned with further investigations and analysis of this virus.

## Isolation of the Agent

While studying the causes of death of the animals at the Anthropoid Ape Research Foundation at Dania, Florida, 3 cases of interstitial myocarditis were encountered. The first occurred in November, 1944, in an adult male gibbon. This animal had been placed in a prominent cage in the farm where many visitors were in fairly close contact with it for several weeks prior to its demise. However, for 10 days before death it had been fairly well sheltered from visitors. No signs of illness were observed until death occurred suddenly and unexpectedly. Autopsy revealed a dilated heart, a slightly blood-tinged pericardial effusion, bilateral hydrothorax, and marked pulmonary edema. An intense interstitial myocarditis was found on histologic examination. No gross or microscopic lesions were found in the brain. The spinal cord was not examined. No attempt was made to recover the etiologic agent.

The second case was encountered in February, 1945, in a well developed, well nourished, 5-year-old male chimpanzee. This animal had been displayed at the entrance to the farm where it was in close contact with visitors, many of whom petted the animal. One morning this chimpanzee vomited its breakfast and died suddenly. Late in the afternoon the body was placed in a refrigerator at about 40° F. The following morning it was examined. The pertinent findings were massive bilateral hydrothorax with slightly pink but clear fluid, distention of the pericardial sac with clear fluid, wide dilatation of all chambers of the heart, and marked bilateral pulmonary edema. Microscopic examination of the heart revealed many minute foci of necrosis with an infiltra-

tion of lymphocytes and scattered polymorphonuclear leukocytes, and pyknotic nuclear structures. Chest fluid and a saline suspension of ground spleen were inoculated into mice and the virus was thus recovered.

The third case of anthropoid myocarditis was found during October, 1945, in a 6-year-old female chimpanzee which had been on exhibition in the same location as the second case. This animal appeared ill for a period of 5 days with edema of the face, subnormal temperature, and dysphagia, and terminally showed considerable cyanosis and dyspnea. Forced feeding and intravenous fluids failed to alter the gradual decline of the animal to its death.

The body had been refrigerated for more than a day at the time of examination. The heart was flabby, weighing 200 gm., the right ventricle was markedly dilated, and on section many grayish discolored, semi-softened areas were present in the myocardium of both ventricles. Bilateral hydrothorax of 500 cc. on the right and 550 cc. on the left, and a marked hemorrhagic pulmonary edema were present. Microscopic examination of the heart showed extensive necrosis and diffuse round-cell infiltration. In many areas the individual fibers were fragmented or entirely replaced by the inflammatory infiltration consisting of lymphocytes, mononuclear wandering cells, and polymorphonuclear leukocytes. The brain and spinal cord revealed no gross or microscopic abnormality. Mice inoculated with this pleural fluid failed to develop any of the typical lesions.

The etiologic agent was recovered from the second case by inoculating two groups of 5 mice, one group with chest fluid and the other with spleen suspended in saline solution. All 10 animals died following paralysis of the hindquarters. A suspension of lungs, hearts, and spleens from these animals was inoculated into 10 other mice intravenously and intracranially. These animals were all paralyzed on the 4th day and dead by the 8th day. These two series of experiments were repeated by inoculation with the original fluids, and the organs from the second series suspended in saline solution formed the basic inoculum with which all subsequent studies were conducted. The virus from the second group was passed through 10 successful mouse passages before it appeared to lose its potency to any degree. The mice all followed a very characteristic course manifested by paralysis on the 4th or 5th day and death on the 7th to 10th day.

### Types of Lesions

In order to study the chronologic development of the disease, a group of 26 mice was inoculated simultaneously and the tissues studied

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microscopically at various intervals. The animals appeared quite well for 2 days; then on the 3rd day many developed rough coats. On the 4th and 5th days approximately one-half developed paralysis of the hind legs and by the 9th day all except 2 had developed paralysis. The paralysis increased in severity until the mice lost all function of the hindquarters and dragged them behind the rest of the body, as in the paralysis caused by the Theiler virus.[12] Finally most of the trunk muscles were involved. In early experiments most animals died by the 8th day. In this series 9 were dead by the 8th day and some lived as long as 37 days before being sacrificed. The cardiac lesion varied with the period of inoculation. The earliest microscopic change was a mild perivascular monocytic infiltration in the heart muscle. About the 6th day small necrotic foci appeared in the myocardium with monocytic infiltration of the surrounding areas. The muscle fibers were separated by edema fluid and cellular infiltrates. Three days later, in addition to the early inflammatory changes, necrosis became more extensive and fibroblasts began to appear. In small foci the muscle was entirely replaced by mononuclear cells and fibroblasts. This was the usual condition of the myocardium in mice succumbing to the virus. Animals sacrificed or dead on the 9th or 10th day frequently showed marked involvement of the auricles. Hearts of animals which survived to the 11th or 12th day had lymphocytes and fibroblasts in increasing numbers. The heart muscle in a period from the 8th to 12th day was extensively involved, frequently throughout the whole myocardium. Calcification was seen as early as 10 days and was very common after the 12th or 13th day, appearing as small granules in the necrotic foci. These granules of calcium usually were arranged in the pattern of the necrotic muscle fibers and frequently appeared while an acute inflammatory reaction was still in progress. After 20 days fibroblastic replacement and calcification were often the only remnants of the disease. It was interesting to note that if a mouse survived to the 12th day, recovery was the rule.

The same inoculum produced a somewhat different process in guinea-pigs. Thirteen healthy mature guinea-pigs were injected with 0.1 cc. of egg inoculum and sacrificed at the indicated periods. Most escaped clinical stigmata of the disease entirely; only one died and that death followed delivery of a small fetus. Seven showed advanced microscopic myocardial changes and 4 had slight or questionable infiltrates. The earliest lesion was seen at 4 days and minute changes were seen as late as 92 days. In temporal spread, the heart lesions were much more persistent and prolonged than in mice. Whereas the acute phase rarely was seen after 6 to 18 days in mice, it often was seen as late as 30 to 35

days in guinea-pigs. The microscopic picture of the heart was similar to that in mice. Foci of necrosis were not as marked but the inflammatory infiltration of the muscle was as widespread and calcification of muscle fibers as distinct.

Hamsters were susceptible to the virus and developed a disease similar to that seen in mice. Four hamsters were inoculated with mouse organs suspended in saline solution. In 10 days they had rough coats, paralysis of the hind legs, and appeared quite ill. Despite the apparent severity of the disease none died and only 2 showed myocarditis when sacrificed following recovery from the paralysis. Two attempts to pass the virus from these hamsters to other hamsters were unsuccessful.

Rabbits were not very susceptible to the virus. Twenty-one rabbits were inoculated with the original chest fluid, a suspension of original ground spleen, various suspensions of mouse organs and egg inocula. Two of the rabbits which were inoculated with the original chest fluid had myocardial changes. One showed areas of necrosis and infiltration with polymorphonuclear leukocytes and large monocytes; the other showed granular degeneration of the myocardial cytoplasm. Three other rabbits had slight perivascular lymphocytic accumulations but exhibited no definite myocardial changes. One rabbit had lipomatosis destruens of the heart after paralysis of the hindquarters which varied in intensity and finally cleared. Detailed microscopic study of the central nervous system did not reveal any change to account for the paralysis. Eleven of these rabbits had serial electrocardiograms. Several showed transient T-wave changes, but no persistent defects could be followed.

Two groups of rats were injected with different potent inocula without the development of either paralysis or cardiac lesions.

### Lesions of Other Organs

The cardiac lesions received special attention in this study and were taken as the criterion of activity of the virus. However, lesions of organs other than the heart were encountered occasionally. The brain, liver, and lungs were examined routinely. The spleen and kidneys also were sectioned in a majority of the cases. Of the organs other than the heart, the central nervous system was affected most frequently. Myelitis was most marked in the period from 4 to 10 days following inoculation when the paralysis was most severe. Sections through the spinal cord at this time showed extensive destruction of all of the outer neural elements with invasion of mononuclear cells and a few polymorphonuclear leukocytes which frequently clumped beneath the meninges. The ganglion cells apparently were not involved by the ex-

tensive myelitis. In this period and somewhat later, foci of encephalomyelitis could be seen in the brain. These areas were frequently beneath the meninges and the outer portion of the brain where they were manifest by groups of mononuclear cells. Frequently there was perivascular cuffing of adjacent vessels. No encephalitis was encountered in the guinea-pigs.

The lungs revealed a variety of minor lesions. Hyperemia was encountered occasionally and often it was accompanied by pulmonary edema which was quite irregular in its distribution. A round-cell thickening of the intra-alveolar septa was encountered and a very small

TABLE I

*Total Injections*

| | Ascitic | Saline | Ascitic-saline | Serum-saline | Glycerinated saline | Other* |
|---|---|---|---|---|---|---|
| Mice | 73 | 52 | 53 | 34 | 14 | 28 |
| Guinea-pigs | 15 | | 21 | 2 | 4 | 2 |
| Rabbits | 7 | 7 | 5 | 2 | 2 | |

* This group includes several animals which were inoculated with pleural fluid and tissue fluid.

proportion of the animals showed bronchopneumonia in addition to the interstitial pneumonic reaction. This interstitial reaction with edema was seen also in guinea-pigs within 3 weeks of inoculation but was not seen later. Occasionally the spleen was grossly enlarged to two or three times the size encountered in control animals. Microscopic examination of the spleen often showed hyperplasia of the lymphoid follicles. A few mice and guinea-pigs had small foci of round cells collected in the medullary portion of the kidneys and interstitial nephritis was seen in 2 guinea-pigs. The livers occasionally showed moderate venous congestive changes and rarely cloudy swelling was present also.

## VIRULENCE OF THE AGENT

### *Media*

Several different media were used to store the virus (Table I). The isolation was accomplished with saline suspensions; however, over a period of 10 days' storage the saline suspensions lost some of their potency. Berkefeld or Seitz filtrates in ascitic or 10 to 50 per cent saline-ascitic mixtures retained potency longer than the saline suspensions. A 10 per cent saline-serum mixture proved as effective as ascitic combinations. Buffered glycerinated saline solution proved an effective carrier in a limited number of inoculations. A summary of inoculations in mice follows:

Nineteen expected potent ascitic suspensions produced myocarditis in 17 groups.

Ten expected potent saline inoculations produced myocarditis in 8 groups.

Eight serum-saline expected potent inoculations produced myocarditis in 8 groups.

Six expected potent ascitic-saline inoculations produced myocarditis in 6 groups.

Three glycerinated saline expected potent inoculations produced myocarditis in 3 groups.

Six expected nonpotent inoculations produced no myocarditis.

### *Controls*

Numerous control groups were studied. Following the isolation of the virus, organs from 5 other anthropoids were inoculated into mice in the same manner used in isolating the agent. Five groups of 4 mice each were inoculated. None of these developed paralysis nor were any myocardial changes noted at autopsy. Two more groups of mice were inoculated with ground heart suspensions from 2 patients, one dying of acute interstitial myocarditis and the other of rheumatic myocarditis. These animals did not have any demonstrable lesions when sacrificed. Three groups were injected with sera from persons suspected of having influenza without the development of any signs of illness or lesions at autopsy.

Various mice were examined at random from the colony and myocarditis was not encountered in any uninoculated animals.

## Character of the Agent

The nature of the agent became apparent when a suspension of it could be passed through Seitz and Berkefeld N filters without loss of potency. Following the second passage through mice, attempts were made to culture organisms from the suspension following filtration. The inocula from 3 successive passages were cultured on all ordinary media for bacteria and yeasts. All of these proved sterile. Only a few passages were made without the virus being passed through a Berkefeld N candle, a Seitz filter, or a Swinney modification of the Seitz filter.

A suspension of mouse hearts in serum-saline solution heated to 70° C. for 20 minutes and potent amniotic fluid heated to 70° C. for 20 minutes were injected intravenously and intraperitoneally into 2 groups of 4 mice each. Neither of these groups developed characteristic lesions. A third group of 6 mice was injected, 2 intravenously, 2

intraperitoneally, and 2 intracranially, with potent ascitic saline suspension heated to 56° C. for 20 minutes. One inoculated intravenously and one inoculated intraperitoneally developed transient paralysis from the 4th to the 6th day. On the 8th day all were paralyzed. None died and at autopsy only 2 had cardiac lesions and these were only moderate.

Due to the limited facilities at hand, the material was stored in a refrigerator freezing unit which maintained a fairly constant temperature at —19° C. The agent retained its potency when stored in the various media for periods as long as 2 months, as indicated by the development of paralysis, myocarditis, and encephalitis following inoculation. Longer periods could not be determined because of mechanical difficulties.

The routine methods of inoculation were intravenously at the base of the tail or intraperitoneally. Intracranial inoculations were used frequently at the beginning of this study, but as they caused rapid paralysis and death before the cardiac lesion was fully developed, other portals seemed more suitable. Nine mice were inoculated by applying the inoculum to the nasal orifices and forcing the mice to breath through the material. All of these mice showed the most advanced myocardial lesions, following a typical course with paralysis. Eight other mice were inoculated in the same manner except that nasal washings from a paralyzed mouse were used to carry the virus; 5 of these developed cardiac lesions, while 3 showed no clinical signs of the disease.

The ability of the agent to develop immune bodies was demonstrated by a positive protection test using the sera of mice recovered from paralysis combined with potent virus at the time of inoculation. Sera from 2 rheumatic fever patients afforded no protection in 2 mouse protection tests. Yellow fever vaccine injected 2 weeks prior to inoculation provided no protection for 6 mice.

The virus was apparently widespread since it could be transmitted by suspensions of brain, heart, kidney, and spleen, or by nasal secretions. Moreover, its potency did not vary markedly from organ to organ.

The allantoic sacs of chicken eggs were used in several passages. One group of 4 7-day-old eggs opened on the third day following inoculation and passed through a Berkefeld filter proved to be the most potent inoculum. This suspension was used extensively. It produced lesions in mice regularly, but could not be successfully transmitted through eggs again. Two other groups of 7-day-old eggs were successfully inoculated with development of potent allantoic fluids. Five other such groups did not develop the virus in a transmittable form.

## Comment

Isolated myocarditis is described by Saphir [13] in an exhaustive and admirable review as exhibiting more or less diffuse inflammatory changes in the myocardium of wide variety and of various causes, having in common principally an isolated involvement of the myocardium by a nonspecific lesion without inflammatory change of the endocardium or the pericardium. The myocardial lesions described by various observers as interstitial myocarditis differed from animal to animal and from one etiologic agent to another. The lesions noted by these authors varied from round-cell infiltration, often perivascular in distribution, to advanced inflammatory reactions with necrosis and polymorphonuclear infiltration.

The myocarditis produced by the virus isolated by us [11] from a chimpanzee varied considerably during different stages of the illness. In mice the first stage observed was slight myocardial perivascular round-cell infiltration, which occurred at about the same time as the onset of paralysis. In the period from 6 to 10 days after inoculation, necrosis of heart muscle and polymorphonuclear infiltration appeared. Calcification appeared at 12 to 14 days with a patchy distribution of fine granules, which seemed to coalesce in the later stages when fibroblastic replacement of the myocardium was developing. The widespread interstitial reaction, occasionally extending from the endocardium to the pericardium and often occurring in both auricles and ventricles, was a characteristic reaction with the chimpanzee agent. This agent also had a primary or secondary affinity for the central nervous system in the smaller rodents, and myelitis of the spinal cord was a consistent finding in paralyzed mice.

Warren and Smadel [14] have maintained the virus by intracerebral inoculations and have found it highly neurotrophic. In mice, encephalitis developed rapidly and some died within 24 hours. The agent was quite potent when inoculated either intraperitoneally or intracerebrally in extreme dilutions. As in my experiments, myocarditis was not observed in the rapidly dying animals but extensive focal necrosis was found when the illness ran a more prolonged course. The guinea-pigs inoculated by Warren and Smadel did not develop myocarditis but showed only a febrile reaction following intracranial inoculation. Guinea-pigs, rabbits, and a rhesus monkey developed specific antibodies during convalescence. Attempts to establish immunologic relationships between this virus and 15 known viruses were unsuccessful in their hands.

In my experiments, the site of inoculation, with the exception of the intracranial route, did not appear to affect the lesion produced by this

virus. That the method of inoculation possibly affected the localization of the lesion is further suggested by the fact that myocarditis was produced in 17 of 22 guinea-pigs in my series inoculated intraperitoneally and intravenously, whereas it was absent in those inoculated intracranially by Warren and Smadel.[14]

A major concern in my early experiments was that perhaps the inoculations had activated a latent virus in the mouse colony, since the paralysis seen in these animals resembled that described by Theiler [12] in spontaneous encephalomyelitis of mice. However, it was suggested that these were different agents because of the fact that the chimpanzee agent could be transmitted by many different methods and not only by the intracranial and intranasal routes as was observed with the Theiler virus, and also because of the rapidity with which paralysis developed in my animals. Later experiments at the Army Medical Center showed that there was no demonstrable immunologic relationship between the chimpanzee agent and Theiler's virus.

Despite the ready passage of the chimpanzee agent into mice when inoculated by a variety of methods, neither myocarditis nor encephalitis was seen in any control animal. In 9 injected groups, a control uninjected mouse was placed in the same cage with the experimental mice inoculated with the agent. None of these showed signs of disease, nor were any microscopic lesions found in these animals at necropsy. Thirty-nine other mice, which were either inoculated or injected with nonpotent material, did not develop any stigmata of the disease.

The isolation of an agent from an anthropoid ape gave rise to the belief that perhaps this was the etiologic agent of interstitial myocarditis in man. This impression was somewhat strengthened when a member of our laboratory personnel developed signs and symptoms of cardiac disease with an elevated sedimentation rate and slight transient electrocardiographic changes. The electrocardiographic tracing reverted to normal in 2 days and the sedimentation rate decreased slowly. However, other members of the laboratory who were subjected to more extensive exposure remained healthy. Neutralization tests performed on 4 people handling these sick anthropoids and working with the virus were negative. One chimpanzee, which had been next to a chimpanzee with myocarditis, showed a small amount of neutralization antibody. This suggests that the chimpanzee agent has little or no pathogenicity for man.

## Summary

1. An agent was isolated from a chimpanzee dying of interstitial myocarditis which produced myocarditis and encephalitis in mice and hamsters and myocarditis in guinea-pigs.

2. This virus produced a range of cardiac lesions from slight perivascular lymphocytic infiltration to advanced myocardial necrosis and polymorphonuclear infiltration.

3. The agent was found to be potent and specific when introduced intravenously, intraperitoneally, subcutaneously, intracranially, and by intranasal instillation. It seemed to be widespread in various organs since it could be transmitted by suspensions of a variety of viscera.

4. This virus has not been identified with any of a variety of known viruses by various biologic tests.

5. The morphologic findings in the heart in this disease duplicate to a remarkable degree the myocardial lesions found in human heart muscle in several virus diseases in patients in whom clinical manifestations were observed prior to death.

Recently Smadel (Smadel, J. E., Medical Department Symposium, Army Medical Center, Washington, D.C., June 5, 1947) reported that stored frozen sera collected from a group of soldiers with "three day fever" in Manila in December, 1945, gave positive results in neutralization tests for this agent. During the course of the illness the antibody titer increased and was highest during convalescence.

I wish to thank Dr. Ferdinand C. Helwig for his guidance and suggestions, and the United States Army Institute of Pathology for the photomicrographs.

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## DESCRIPTION OF PLATES

### PLATE 19

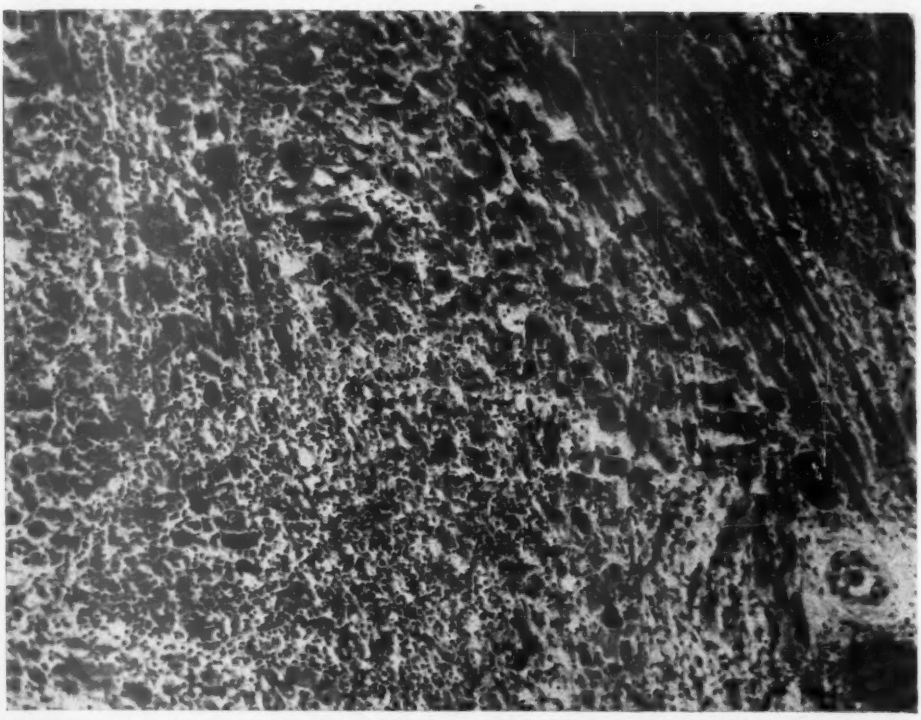
FIG. 1. Heart of an adult male gibbon. There is widespread involvement of the myocardium with areas of necrosis. Hematoxylin and eosin stain. × 110. (Army Institute of Pathology accession no. 145645; neg. 89608.)

FIG. 2. Higher magnification of a field from Figure 1. Hematoxylin and eosin stain. × 350. (A.I.P. acc. 145645; neg. 89609.)

1

2

1



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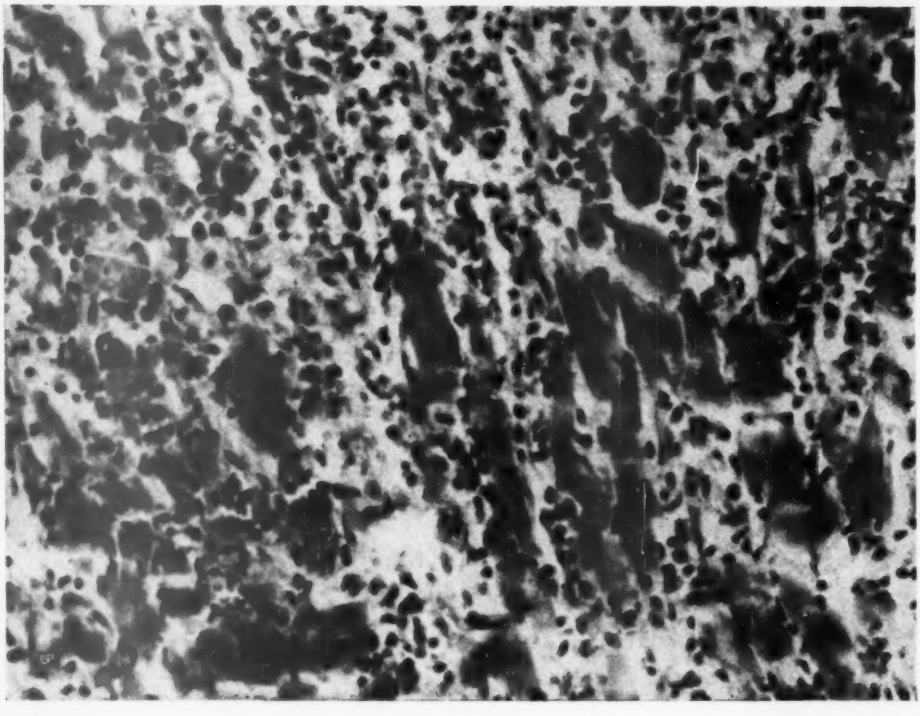


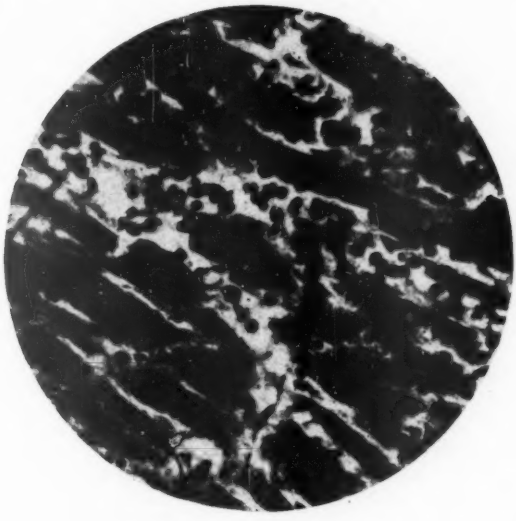
Schmidt Virus Myocarditis

PLATE 20

FIG. 3. Five-year-old male chimpanzee. The myocardium is invaded with lymphocytes. Hematoxylin and eosin stain. × 380.

FIG. 4. Mouse heart on the 6th day of inoculation. Edema and round-cell infiltration are present. Hematoxylin and eosin stain. × 350. (A.I.P. acc. 145645; neg. 89603.)

3



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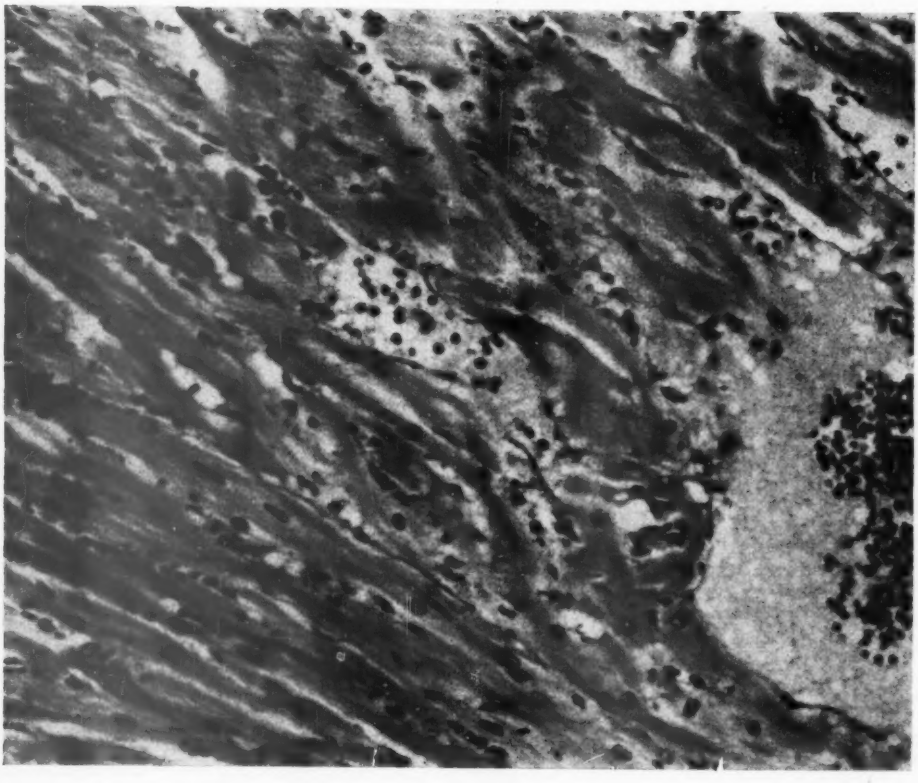


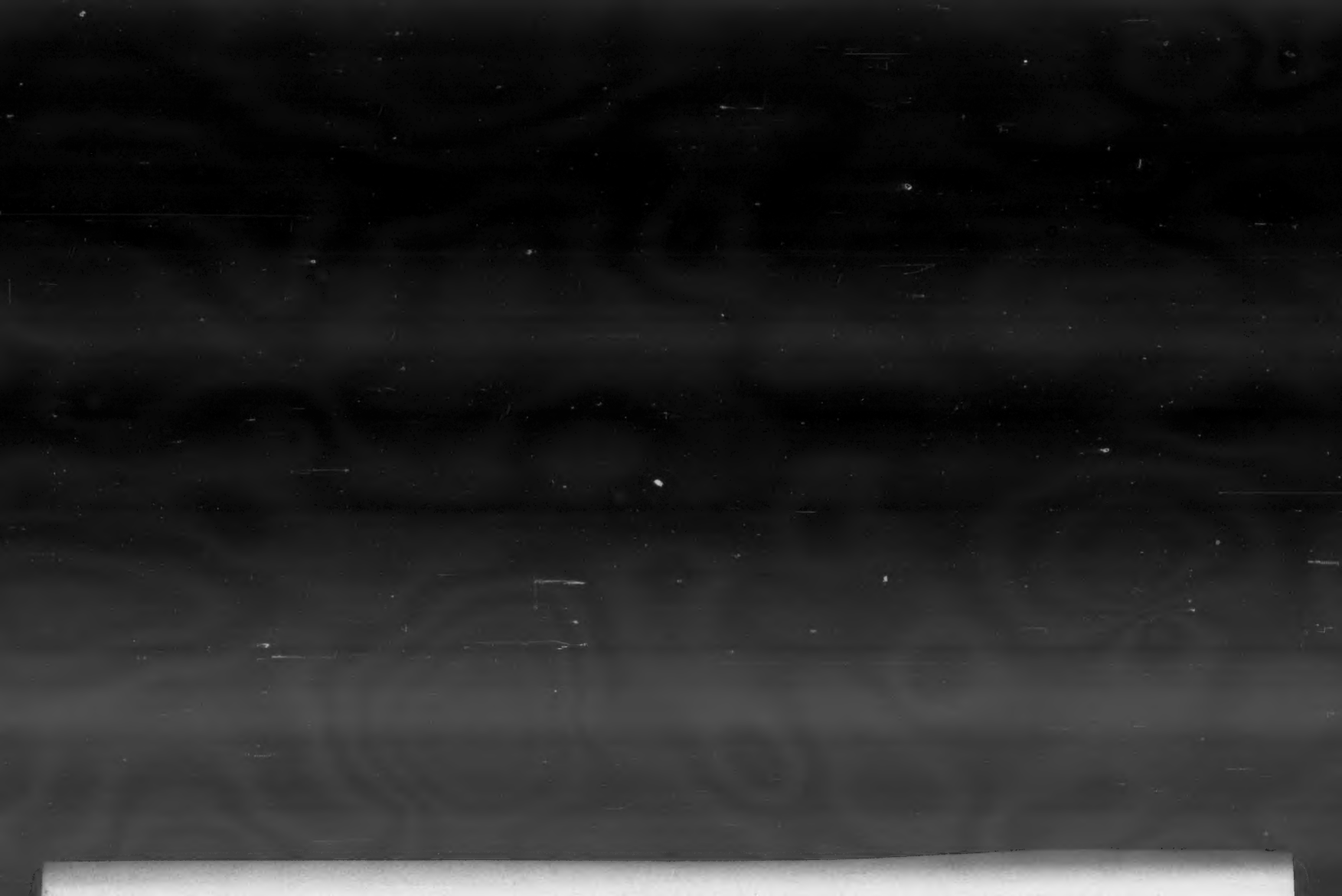
Schmidt Virus Myocarditis

PLATE 21

FIG. 5. Mouse heart on the 10th day of inoculation with mononuclear infiltration and early fibroblastic replacement. Hematoxylin and eosin stain. × 350. (A.I.P. acc. 145645; neg. 89601.)

FIG. 6. Mouse heart on the 10th day following inoculation, illustrating the marked auricular involvement at this stage. Hematoxylin and eosin stain. × 110. (A.I.P. acc. 145645; neg 89602.)

FIG. 7. Mouse heart on the 12th day following inoculation. Many fibroblasts are present. Hematoxylin and eosin stain. × 110. (A.I.P. acc. 145645; neg. 89647.)



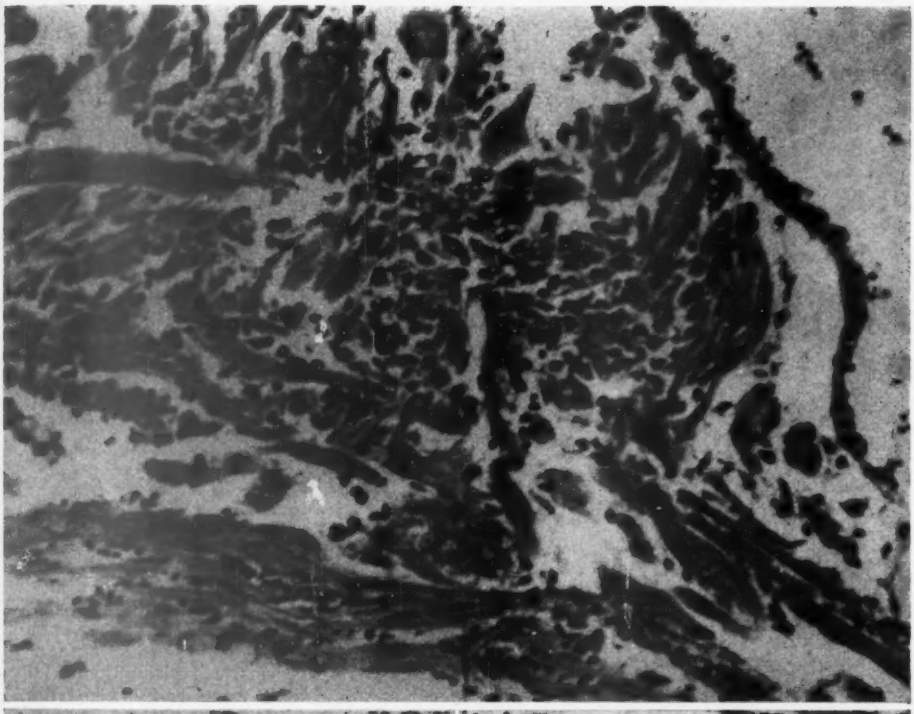


PLATE 22

FIG. 8. Mouse heart on the 13th day following inoculation. Calcification of the necrotic muscle fibers has taken place. Hematoxylin and eosin stain. × 110. (A.I.P. acc. 145645; neg. 89600.)

FIG. 9. Guinea-pig heart 17 days following inoculation. The marked calcification is characteristic of the reaction in this animal. Hematoxylin and eosin stain. × 350. (A.I.P. 145645; neg. 89593.)

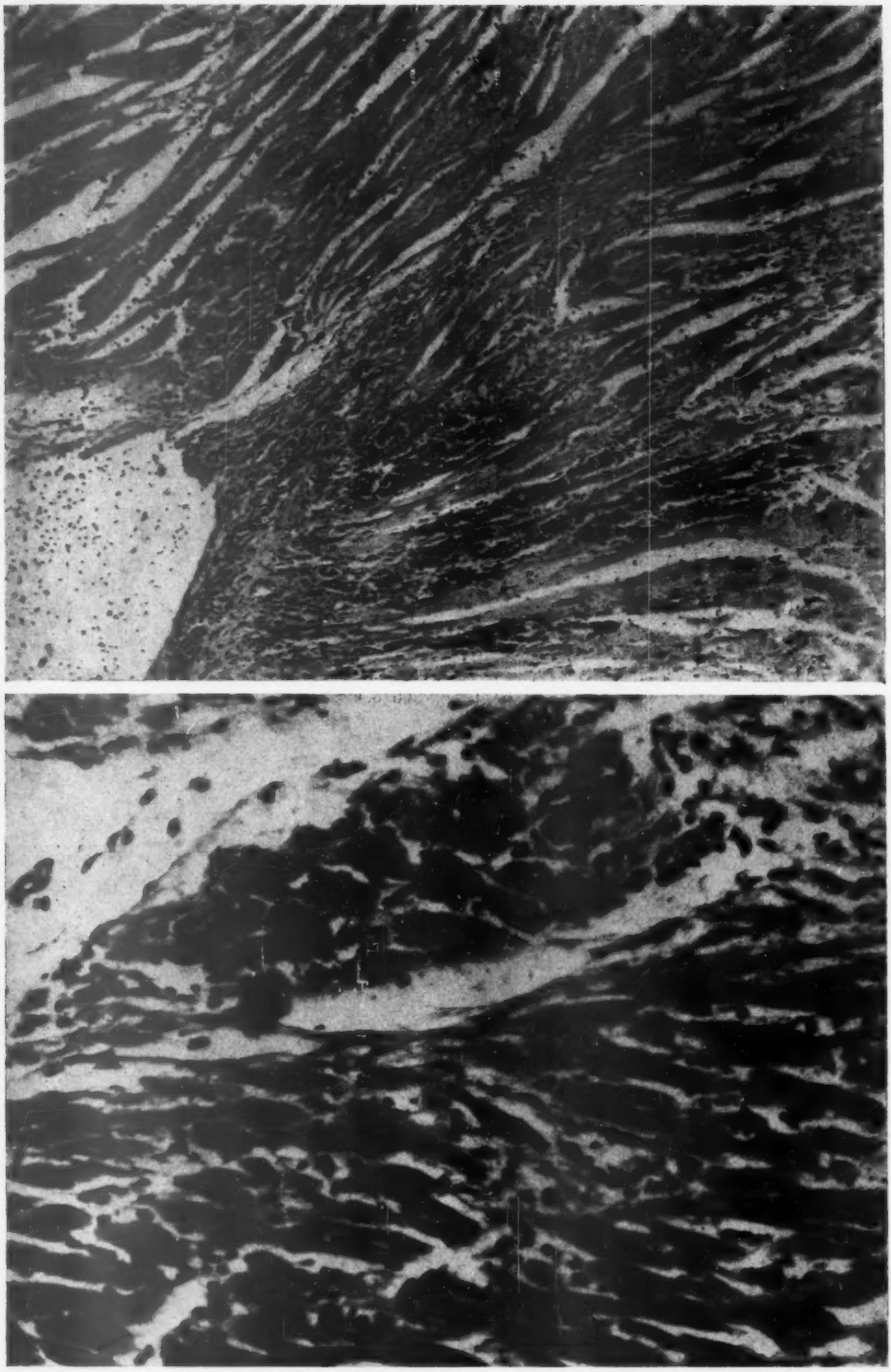
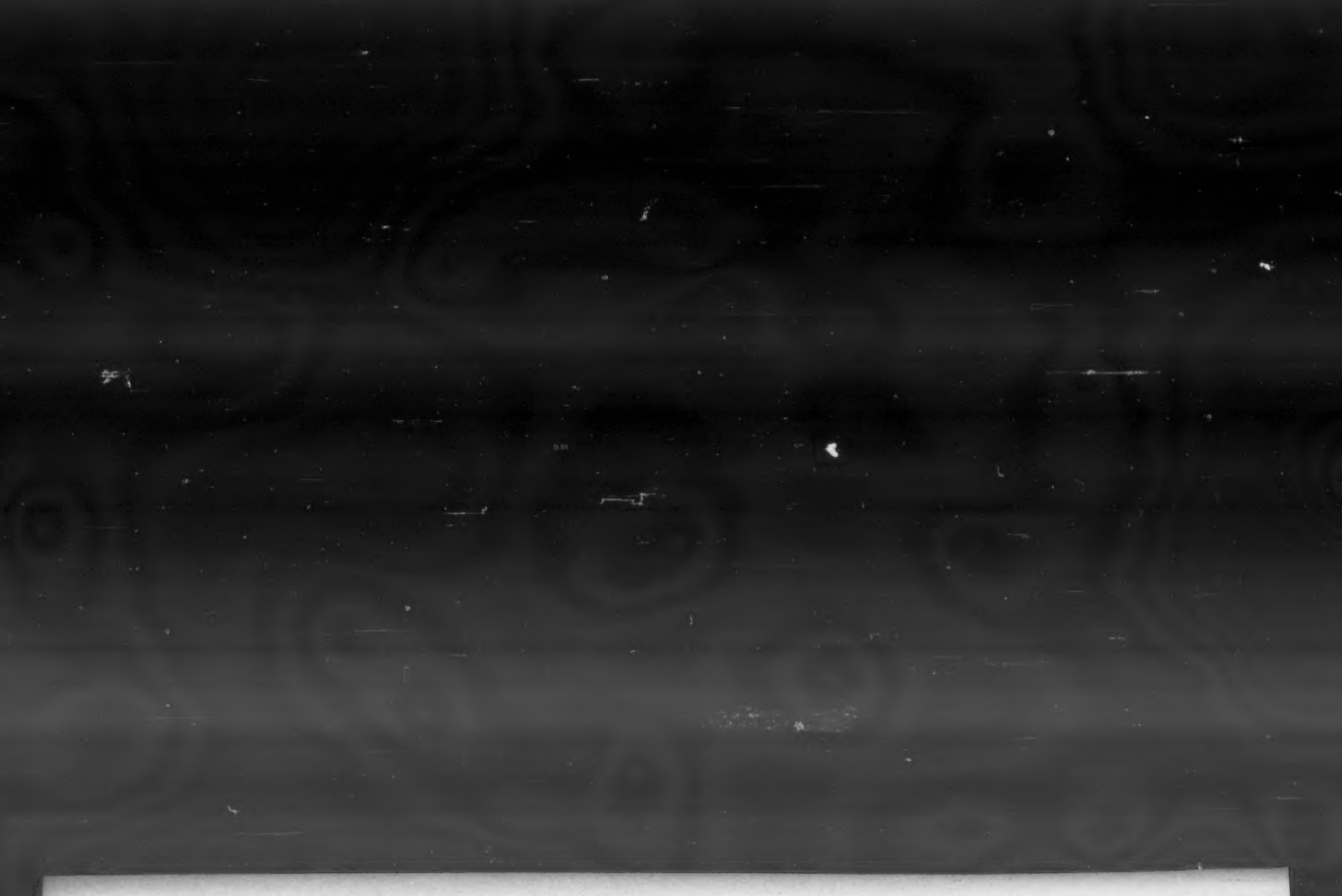
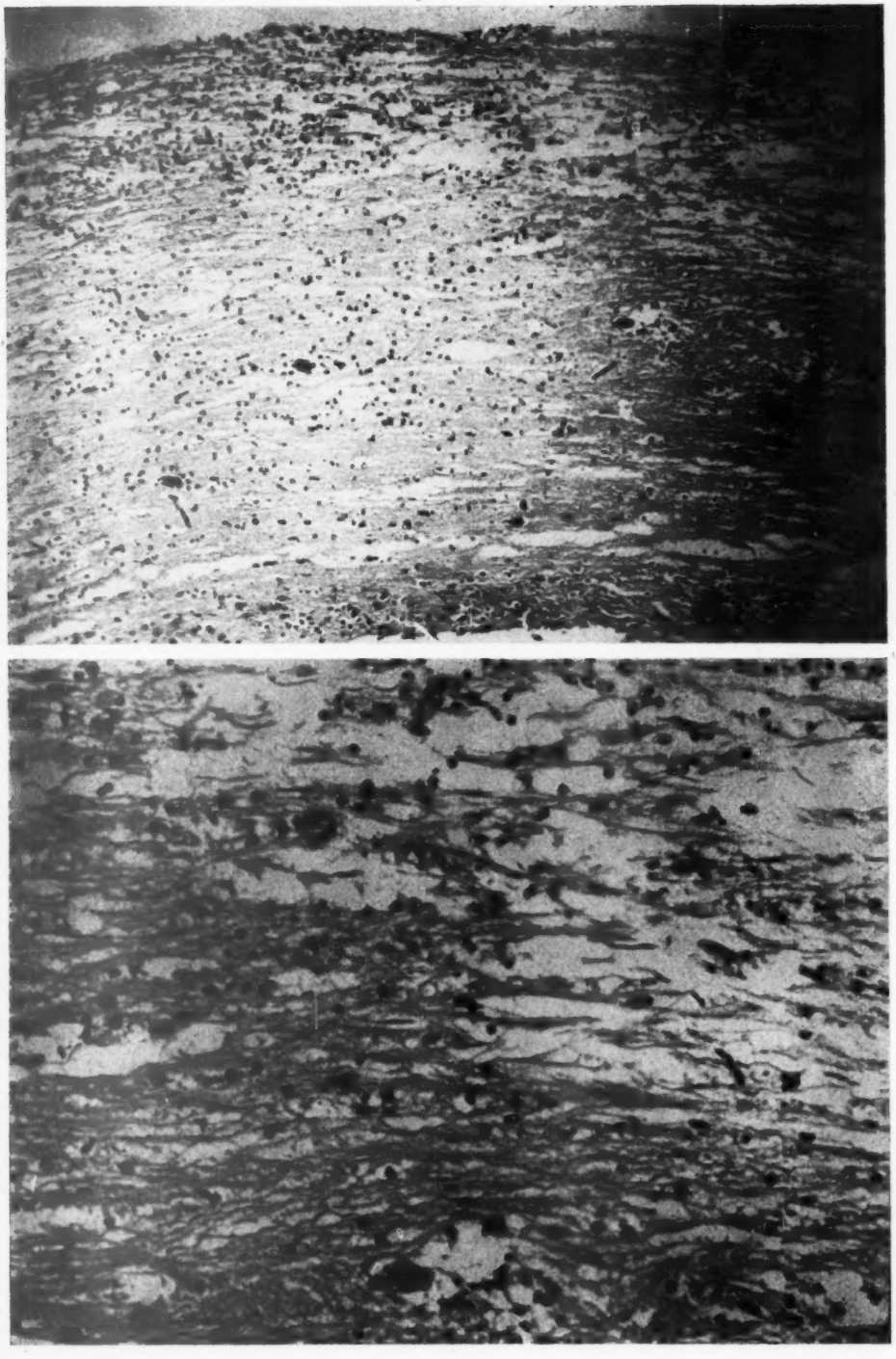


PLATE 23

FIG. 10. Spinal cord of a mouse which died on the 10th day following inoculation. The peripheral myelitis with perivascular cuffing is evident. Hematoxylin and eosin stain. × 110. (A.I.P. acc. 145645; neg. 89649.)

FIG. 11. Higher magnification of a field from Figure 10. Hematoxylin and eosin stain. × 350. (A.I.P. acc. 145645; neg. 89648.)





Schmidt Virus Myocarditis

# HISTOLOGIC CHANGES IN RICKETTSIALPOX *

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A new rickettsial disease was recognized in July, 1946, following an outbreak of an epidemic in one of the boroughs of New York City (Shankman [1]). This mild febrile disease with an eruption not unlike atypical chickenpox was named "rickettsialpox."

Greenberg and Pellitteri [2] of the New York City Health Department identified the disease as a separate entity. Huebner, Stamps, and Armstrong [3] of the National Institute of Health established the rickettsial etiology of the disease and developed a diagnostic complement-fixation test. Huebner, Jellison, and Pomerantz [4] traced the vector of the infection, a rodent mite (*Allodermanyssus sanguineus*), and the reservoir, the domestic mouse. The clinical picture was reported by Shankman [1] and by Greenberg, Pellitteri, Klein, and Huebner.[5]

The disease is characterized by fever of about 1 week's duration and a maculo-papulo-vesicular rash. The eruption may be present on any part of the body, including the face, but the palms and soles are rarely affected by the rash. Occasionally the lesions may appear on the oral mucosa. Many patients suffer from photophobia and sore throat. Enlargement of lymph nodes and spleen occurs in some cases.

The majority of patients also have an *initial lesion* which develops at the location of the bite of the mite. It is usually found in some area covered with clothing or with hair (Shankman [6]), but none of the patients has felt the bite. The lesion is a dark red papule from 0.5 to 1.5 cm. in diameter at the base, with a deep-seated vesicle filled with fluid which is clear at first, but later becomes cloudy. The vesicle dries to a black eschar. The initial lesion persists for about 3 weeks. A scar remains after the sequestration of the eschar. Clinical manifestations of the disease begin 1 week after the appearance of the initial lesion.

## REPORT OF CASES

Biopsies of skin were made on 6 patients, and a lymph node was excised in one case. Three patients who were admitted to the Willard Parker Hospital during the original investigation of the disease had resided, permanently or temporarily, in the area where the disease was prevalent. Three other patients who became ill several months later lived in other parts of the city, had never been in the locality of the epidemic outbreak, and had had no contact with anyone having a disease with an eruption.

* Received for publication, August 9, 1947.

### *Case 1*

M. K. was a white female, 20 years old. Her mother became ill on July 7, 1946, and recovered 10 days later. The patient noticed a lesion resembling an insect bite on her left shoulder on July 15. On July 22 she had a headache; on July 23, fever, loss of appetite, and pains in the muscles. On July 24 she had shaking chills, and her temperature rose to 104° F. During the next 2 days she developed photophobia, sore throat, and a rash on the neck, body, and extremities, appearing in crops. She entered the Willard Parker Hospital on July 24, volunteering to cooperate in the study of the disease.

Her temperature on admission was 103.2° F.; pulse, 100. The skin was flushed, the conjunctivae were congested. A sparse rash was present on the trunk and extremities. It consisted of irregular erythematous macules gradually rising to a central or eccentric papule surmounted by a minute vesicle sometimes covered with a scab. Enlarged lymph nodes were present in the posterior cervical region and in the right axilla; the spleen was 2 fingersbreadth below the costal margin. A brownish crusted lesion ½ inch in diameter was present on the left shoulder.

*Laboratory Examinations.* On the day of admission a specimen of blood for animal inoculation was obtained by Dr. Robert J. Huebner of the National Institute of Health. The blood count on July 27 showed 5,000 white blood cells, with 43 per cent neutrophils, which on subsequent examinations rose to 8,400 and 8,250 white blood cells, with 47 and 49 per cent neutrophils. The urine on July 27 was free of albumin, but showed a few hyaline casts; on July 29 it was free of casts. The blood culture taken on July 29 was sterile. The Kline test was negative. Agglutinations with typhoid O and H, paratyphoid A and B, and brucella antigens were negative; with proteus OX 19, positive at 1:80. On July 31 the agglutinations were: typhoid O, positive at 1:20; typhoid H, negative; paratyphoid A and B and brucella, negative; *Past. tularensis,* negative; *B. proteus* OX 19, positive at 1:20. Chemical examination of the blood showed normal findings.

On July 28 (seventh day of illness, fifth day of rash) a skin lesion beneath the right breast and a left inguinal lymph node were removed for microscopic examination (Figs. 9 and 10).

*Course in the Hospital.* The temperature went down by lysis on July 28. The rash was gone on July 31 leaving a brown pigmentation, the lymph nodes were no longer palpable, the spleen was less firm, but still palpable. The initial lesion became a reddish brown papule. The patient was discharged on July 31.

The specimen of blood inoculated by Dr. Robert J. Huebner [3] into mice and guinea-pigs produced illness in mice 9 days later. One mouse was killed and was found to have large abdominal lymph nodes and spleen. Passages from these organs produced a similar disease in other mice and guinea-pigs. Rickettsiae were recovered from the yolk sacs of fertilized eggs cultured with the brain of a mouse from the second passage which had been inoculated intracerebrally. Animals inoculated with these yolk sacs developed the same illness as those inoculated with the patient's blood, and an antigen prepared from the yolk sacs gave a highly specific complement-fixation test with a later specimen of blood of this patient in dilution of 1:512, and positive reactions with sera of other patients in the stage of convalescence. A rising titer was observed where repeated tests had been made.

### *Case 2*

H. B., a white male, 34 years old, noticed a red papule on his right shoulder on July 17, 1946, another lesion in the left axilla on July 23, and became ill on July 25. The rash appeared on July 26. The highest temperature was 103° F. He was admitted to the Willard Parker Hospital on July 27. The rash was of the same character as in the first patient. There was a slight enlargement of cervical lymph nodes and a marked enlargement of axillary nodes. The spleen was barely palpable. Biopsy of the skin was performed on July 28 (Fig. 7). The patient was discharged on August 3, with only a few papules left. The complement-fixation test with the M. K. (case 1) antigen 25 days after the onset of illness was positive at 1:320.

### *Case 3*

R. R., a white female, 50 years old, stayed for 10 days in the home of her daughter where three members of the family were ill. She never noticed an insect bite and had no initial lesion. On the tenth day of her visit, on July 25, she had chills and fever. Between July 27 and 30 she developed a rash, first on the neck, later on the abdomen, with tender inguinal lymph nodes. She was admitted to the Willard Parker Hospital on July 30. On July 31 she had papules surmounted by shiny vesicles on the skin, and a nodule on the hard palate. The temperature was 103° F. A papulovesicular lesion from the abdomen was removed for microscopic examination (Fig. 8). The temperature became normal on August 2. She was discharged on August 7.

### *Case 4*

M. T., a white female, 46 years old, living in a part of the city where no cases of the disease had previously been reported, noticed a slightly itchy "lump" over the right scapula on January 21, 1947. On January 26 she developed a rash on the face and felt sufficiently ill to stay in bed. On January 28 she was seen by a physician who considered the possibility of chickenpox, but on January 30 referred her to the Willard Parker Hospital with a diagnosis of typhus fever.

On admission, on January 30, she had a temperature of 103.4° F.; pulse, 130; scattered macules and papules on the face, trunk, and extremities; and a large, deep red, firm nodule over the right scapula, covered with a dry, scaly epithelium. There was cervical and axillary lymphadenopathy, more marked on the right side. On January 31 the temperature was 100° F. A piece of skin containing the initial lesion and a macule of the rash was removed from the region of the right scapula (Figs. 1, 2, and 3). She was discharged on February 4, with a fading rash. The complement-fixation test for rickettsialpox on January 31 was negative; on March 12, positive at 1:32.

### *Case 5*

L. L. was a Puerto Rican female, 23 years old. She had been in the United States for 3 months, living in a house infested with mice, in a part of the city where the disease had not been reported previously. On January 30, 1947, while bathing, she noticed a small pimple in the right inguinal region. On January 31 she had a headache, pain in the neck, temperature, and an eruption on the face. The following day she had a scattered eruption on the body.

On admission to the Willard Parker Hospital on February 2 she had a temperature of 99.4° F., with papules on the face, chest, abdomen, and extremities, some with vesicles. A small papular lesion, scratched open, was present in the right inguinal region. There was no lymphadenopathy. On February 3 her temperature was normal. A specimen was removed for biopsy from a flat papular lesion, 4 mm. in diameter, in the right deltoid region (Figs. 4, 5, and 6). She was dis-

charged on February 10. The complement-fixation test for rickettsialpox on February 4 was negative; on March 31, positive at 1:32.

### *Case 6*

M. B. was a colored female, 44 years old, who lived in a part of the city where rickettsialpox had not been observed previously. She was admitted to the Willard Parker Hospital on April 24, 1947, with complaints of headache, pain in the left side of the neck, malaise, dizziness, mild chills, and fever of 5 days' duration, and of a mild rash on the face and back which had appeared on the preceding day. The temperature on admission was 100.4° F.; pulse, 84. She had a faint generalized erythema with punctate nonerythematous lesions, and sparse erythematous papules, some with whitish centers, over the abdomen. The pharynx was congested, the tongue coated, and the spleen was 2 fingersbreadth below the costal margin. The right inguinal area was slightly tender, but no lymph nodes were palpable. The liver was palpable. The diagnoses considered on admission were rickettsialpox and typhoid fever. Agglutinations with *Bacterium typhosum, Bact. paratyphosum* A and B were negative, with *Bacillus proteus* OX 19, positive at 1:40. On April 25 the patient was afebrile. The spleen was 2 fingersbreadth below the costal margin. A biopsy was taken from a papular lesion. She was discharged on April 29. The complement-fixation test for rickettsialpox on April 28 was positive at 1:8; on May 29, positive at 1:32.

## Material for Biopsy

The material for biopsy was fixed in Regaud's solution for 30 hours, embedded in paraffin, and stained with Giemsa's stain, the technic recommended by the National Institute of Health for the demonstration of the rickettsiae. Additional sections were stained with hematoxylin and eosin.

### *Initial Lesion*

The initial lesion excised from the scapular region of case 4 was a dark red, deep-seated, firm nodule 8 mm. in diameter (Fig. 1). A pustule covered with dry and scaly epithelium occupied the center of the nodule. It measured 5 mm. in diameter and was raised 2 mm. above the base. When the lesion was sectioned, a small amount of yellow fluid escaped and the pustule partly collapsed. The section showed that, while the lesion was deep-seated, the pustule proper was superficial. The corium beneath the pustule was indurated, pale pink, rather moist, and contained grossly visible dilated blood vessels. Microscopically, the pustule was situated entirely within the epidermis. The epithelium was elevated in a vault-like manner; its cells were compressed and showed coagulation necrosis and polymorphonuclear infiltration of the deeper layers. The basal epithelium was present only at the angle of the pustule, and there the intra-epidermal character of the pustule became apparent (Fig. 2). The rest of the basal layer was missing, and the floor of the pustule was formed by the exposed corium. The floor of the pustule was slightly and superficially infiltrated with polymorphonuclear cells at the center of the lesion but showed no necrosis

of the stroma. The pustule was filled with serum, shreds of necrotic epidermis, and polymorphonuclear leukocytes. The capillaries in the deeper layers of the corium were dilated, and some contained polymorphonuclear cells. The endothelium of other capillaries was swollen, bulging or desquamating into the lumen, and occasionally showed mitotic figures. Several smaller capillaries contained pale pink homogeneous material partly occluding the lumen (Fig. 3). Mononuclear cells and occasional polymorphonuclear cells were scattered in the corium and were more densely aggregated near the blood vessels. The mononuclear cells were lymphocytes, mast cells, and peculiar cells with a small amount of dark or light cytoplasm, one or two processes, a large, bulging nucleus, and a dark nucleolus; connective tissue cells and fibroblasts were present also. The mast cells were most numerous at the periphery of the lesion, especially in mononuclear infiltrates surrounding the hair roots and other cutaneous appendages. They were elongated or polyhedral, were densely packed with metachromatic material, and appeared as solid, dark purple masses under low and high-dry magnifications; under the oil-immersion lens, however, the metachromatic granules often could be seen within the body of the cell, and always in its processes. The cells with a small amount of cytoplasm and a bulging nucleus were found to be similar to the lymphoid wandering cell illustrated in Maximow and Bloom's textbook.[7] No plasma cells were seen in the infiltrates.

### *Rash*

Four maculopapular and two papulovesicular lesions were examined. In a maculopapular lesion the epidermis was intact or was slightly thinner than in the adjacent skin, with some scaling on the surface (Fig. 4). In the upper layer of the corium there was an increased number of connective tissue cells. Capillaries, veins, and cutaneous appendages (hair roots, sebaceous and sweat glands, and also arrectores pilorum muscles and nerve trunks) were surrounded by dense collections of cells, but there was no diffuse infiltration of the corium (Figs. 4 to 7). Few cells with vesicular nuclei were present. The mast cells were more numerous than in the initial lesion (from 5 to 15 in a high-power field). In some cases numerous pyknotic nuclei and nuclear fragments were scattered among the cells of the infiltrates. Lymphocytes were numerous, but polymorphonuclear cells were rare; in 2 cases they were eosinophilic. Homogeneous pale pink thrombi or strands of fibrin were seen in some capillaries, and the endothelium of many capillaries bulged toward the lumen. In one case occasional mast cells were found beneath the desquamating endothelium of small veins.

The epithelium of the sweat glands was sometimes hyperchromatic, but showed no desquamation; occasional mast cells invaded the walls of the sweat glands.

In a fresh papulovesicular lesion the vesicle occupied the entire thickness of the epidermis. The greater part of the epithelium forming the thick top of the vesicle showed vacuolization and some disintegration of the cells with fragmentation of the nuclei, but the basal layer was largely intact (Fig. 8). There was no "ballooning" or hyalinization of the epithelial cells as seen in chickenpox. The vesicle contained a small amount of fibrin and a few polymorphonuclear cells. The upper layer of the corium was streaked with elongated nuclei of leukocytes migrating toward the vesicle and contained a thrombosed blood vessel. Perivascular and some diffuse mononuclear infiltrations were present in the deeper layers of the corium beneath the vesicle, but on either side of that area the picture was the same as in maculopapular lesions.

In the healing papulovesicular lesion the vesicle was located in the cornified layer of the epidermis and contained only thin protein material (Fig. 10). The epithelium beneath the vesicle had been restored, but was slightly concave. Periadnexal infiltrates and vascular changes still were present in the corium.

### *Lymph Node*

The lymph node excised from the inguinal region of case 1 was bean-shaped, moderately soft, and measured 8 by 5 mm. The cut section was pale pink and slightly granular. Microscopically, the lymphoid tissue showed nothing remarkable except a few eosinophils here and there and several groups of mast cells. The stroma of the hilus was edematous and contained several small groups of mast cells and scattered cells with large vesicular nuclei similar to those seen in the infiltrates in the corium of the initial lesion (Fig. 9).

## Discussion and Summary

The gross appearance of the initial lesion of rickettsialpox is similar to that of the primary lesion of scrub typhus and of the tache noir of Marseille fever.

No microscopic description of the tache noir seems to be available in the literature, but the microscopic picture of the initial lesion of rickettsialpox closely resembles the histologic findings in the primary lesions of scrub typhus as described by Allen and Spitz.[8] The pustules in these two diseases are identical. The difference in the inflammation of the corium is largely quantitative. The area of polymorphonuclear infiltration of the corium in rickettsialpox is more limited

and more superficial than in scrub typhus, and there is no degeneration of the connective tissue. The vascular changes also are less severe. The cellular infiltrates follow the same perivascular and periadnexal character as in scrub typhus, but the plasma cells are absent and the mast cells are more numerous.

The maculopapular rash of rickettsialpox is microscopically similar to the eruptions of other rickettsial diseases in regard to the character and distribution of the cellular infiltrates, but the infiltrates are much heavier than in any other rickettsial disease. Karyorrhexis may be quite prominent, and mast cells densely packed with metachromatic granules are numerous. Plasma cells are absent. The vascular changes in rickettsialpox closely resemble those of scrub typhus, but are less severe. Incomplete homogeneous thrombi and the absence of arteritis and hemorrhages in the eruption of both diseases distinguish them from typhus, Rocky Mountain spotted fever, and Marseille fever.

The vesicle of the rash is unique in a rickettsial disease. The epithelium at the top of the vesicle shows vacuolization and some disintegration of the cells with karyorrhexis. The basal epithelium is largely intact. The corium immediately beneath the vesicle shows some migration of polymorphonuclear cells and a slight diffuse mononuclear infiltration, but in the papular portion of the eruption which forms the base of the vesicle the changes in the corium are the same as in other papular lesions.

The lymph node showed no necrotic changes characteristic of scrub typhus, but the presence of mast cells in the lymphoid tissue and in the stroma of the hilus was an evidence of some damage to the node.

Although rickettsiae (*R. acari*) had been recovered from the blood of some patients, they have not been found in sections of the skin lesions or of the lymph node.

Rickettsialpox is a mild disease with a uniformly good prognosis, but in the early stage the clinical picture may be confusing, especially in the absence of an initial lesion. The diagnoses of chickenpox, typhus fever, and typhoid fever were considered in some of our patients at the onset of illness.

Several laboratory procedures have been devised as diagnostic aids, but some of them proved to be impractical. Cultivation of the rickettsiae from the blood of the patients is so complicated and protracted as to make it completely unsuitable for diagnostic purposes. The complement-fixation test is the best diagnostic laboratory procedure, but it usually requires at least two specimens of blood taken 3 or 4 weeks apart, if the first test is negative. Microscopic examination of the rash has its place as an aid in the diagnosis of rickettsialpox, especially if

a suspicious disease appears in an area previously free from that disease. The histologic examination of a skin lesion may be completed long before the report on the second specimen of blood is available and will help in securing earlier confirmation of the diagnosis.

I wish to thank Drs. Arthur C. Allen and Sophie Spitz for making their material on rickettsial diseases available for study and for helpful suggestions in the preparation of this paper.

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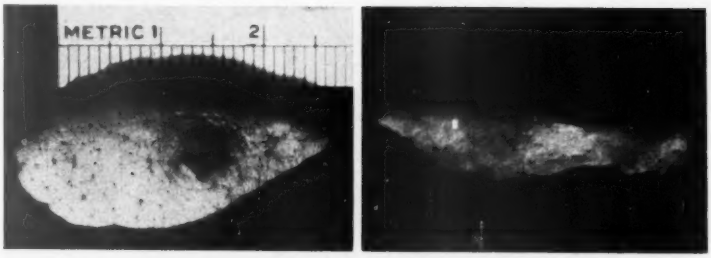
## DESCRIPTION OF PLATES

### PLATE 24

FIG. 1. Case 4. Initial lesion of rickettsialpox, at least 10 days old. The bisected specimen at the right shows the superficial collapsed pustule and the indurated, slightly congested area in the corium.

FIG. 2. Case 4. Initial lesion of rickettsialpox. Angle of the pustule showing the intra-epidermal character of the pustule and loss of the basal epithelium a short distance from the angle. The epithelium of the wall is compressed, and polymorphonuclear cells from the lumen of the pustule invade the necrotic inner layer of the epithelium. Giemsa's stain. × 220.

1

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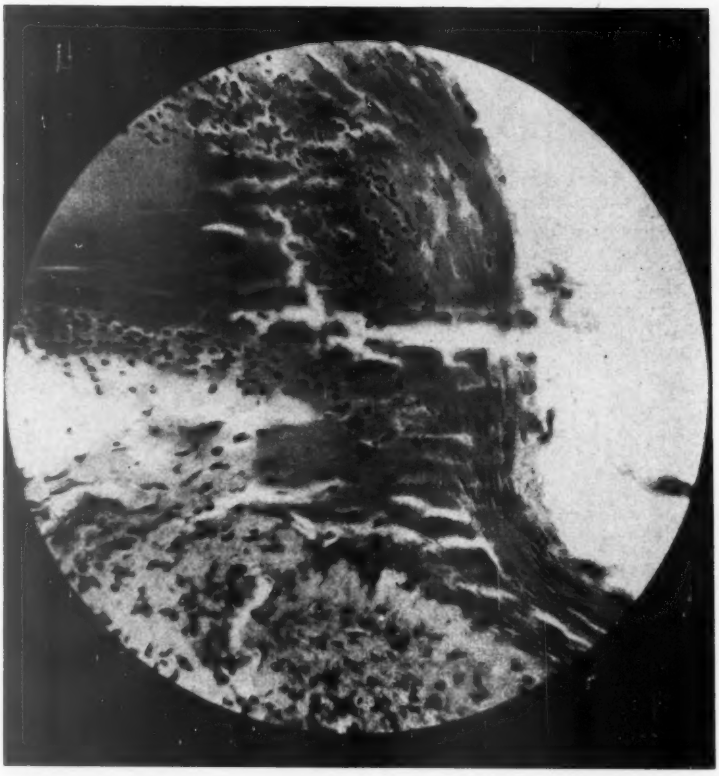


Dolgopol Histologic Changes in Rickettsialpox

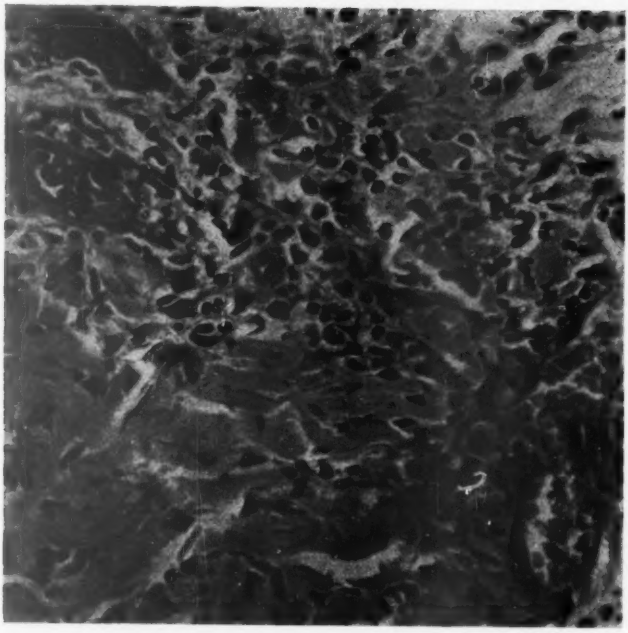
## PLATE 25

FIG. 3. Case 4. Cellular infiltration and congestion of the corium beneath the pustule. Swollen endothelium in the branching capillary; mitotic figure in an endothelial cell in the right lower corner of the field. Homogeneous material and desquamated endothelium in the lumen of the capillary in the upper left corner. Cells with vesicular nuclei adjacent to the capillary; several similar cells in the central portion of the field. Giemsa's stain. × 440.

FIG. 4. Case 5. Papule of rickettsialpox. Slight scaling of the epidermis. Perivascular and periadnexal cellular infiltrates. Mast cells appear as large dark bodies. Giemsa's stain. × 100.

FIG. 5. Case 5. Detail from Figure 4. Homogeneous material partly fills the lumen of a capillary. Nuclear fragments and mast cells are present in the perivascular infiltrate. Mast cells are present also near the arrector pili muscle. Giemsa's stain. × 660.

3



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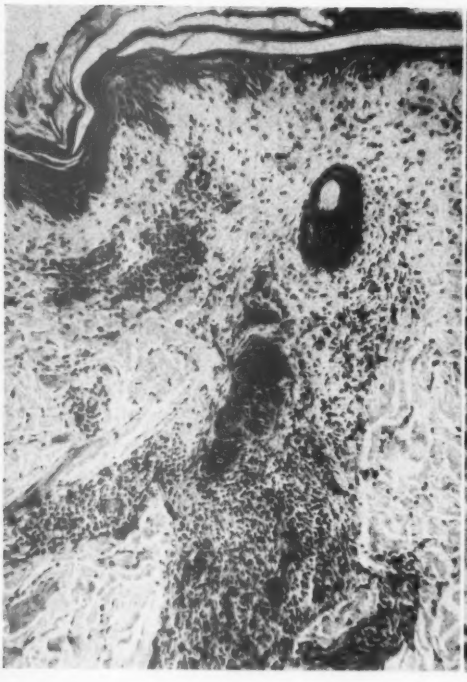

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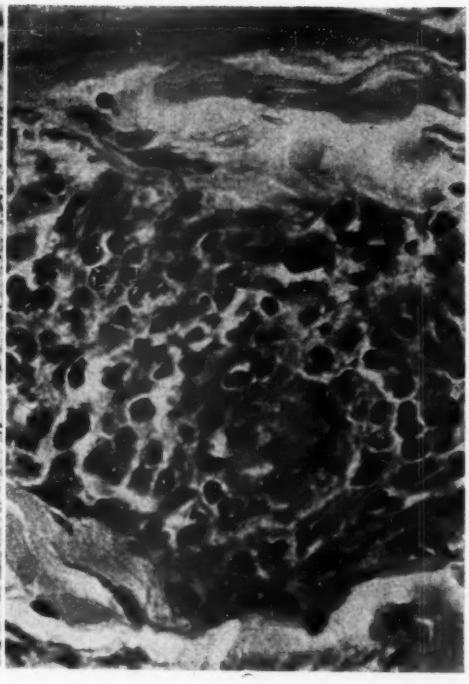

Dolgopol

Histologic Changes in Rickettsialpox

PLATE 26

FIG. 6. Case 5. Cellular infiltrate around a sweat gland in another area of the corium beneath the same papule seen in Figures 4 and 5. Mast cells and nuclear fragments are scattered among the lymphocytes. Giemsa's stain. × 440.

FIG. 7. Case 2. Maculopapular rash of rickettsialpox. Mast cells in the infiltrate around a sebaceous gland and arrector pili muscle. Giemsa's stain. × 440.

FIG. 8. Case 3. Fresh papulovesicular rash of rickettsialpox. Intra-epidermal vesicle covered with a thick layer of epithelium showing hydropic changes and some disintegration of the cells. The basal layer is largely preserved. Some nuclei of migrating polymorphonuclear cells are present in the upper layer of the corium. A slight diffuse mononuclear infiltration is seen in the lower layers of the corium. Hematoxylin and eosin stain. × 100.

e

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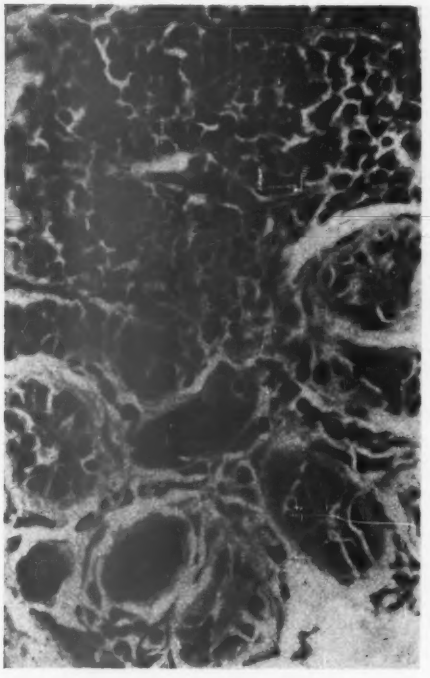

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8

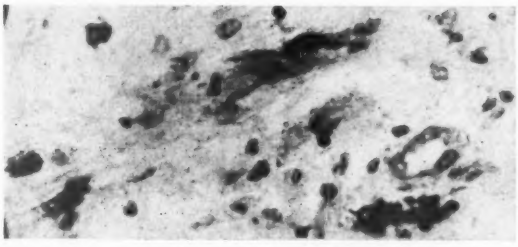
Dolgopol Histologic Changes in Rickettsialpox

PLATE 27

FIG. 9. Case 1. Mast cells in the stroma of the hilus of an inguinal lymph node. Giemsa's stain. × 440.

FIG. 10. Case 1. Drying vesicle of a papulovesicular rash of rickettsialpox. The vesicle is located in the cornified layer of the epidermis. The epithelium at the base of the vesicle is restored to normal thickness. Giemsa's stain. × 100.

9



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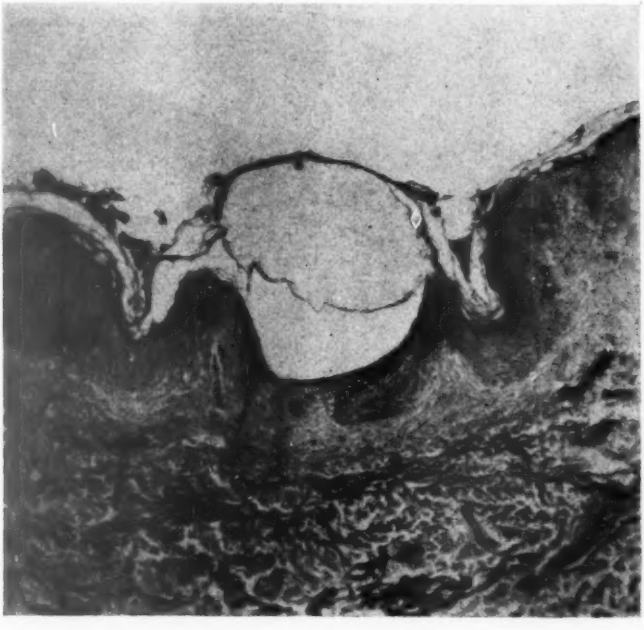

# STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

## X. Effects of Slow Nervous Action of Disuse on the Structure of Nerve Endings, Neurosomes, and Muscle Fibers *

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The morphologic effects of inactivity upon the nerve endings of the neuromuscular apparatus in skeletal muscle have remained an unexplored field. The surgeon empirically has discovered and rediscovered the beneficial effects of early ambulation after an operation. The structural changes, however, in nerve and muscle, produced by immobilization are practically unknown. The knowledge of the pathologic changes induced by disuse in nerve endings may give a clear insight into the normal nervous discharge and the substantial dependence of nerve and muscle.

The experimental study herein reported is a product of the hypothesis that normally there is a discharge of fine neurogenic granules into muscle.[1] Considerable evidence has been presented that under physiologic conditions there is a tenuous and evanescent secretion of very fine neurogenic granules into muscle and that these granules undergo rapid periodic discharge, diffusion, and disappearance by hydrolysis. It is assumed that under the pathologic conditions of disuse, by various methods, the neurogenic secretion accumulates slowly and forms enlarged droplets. These enlarged neurogenic droplets are considered to be the products of retardation in the rate of the periodic discharge, diffusion, and dissolution by hydrolysis. During the early period following the disuse of muscle, a pathologic dissociation may be produced of the neurogenic and myogenic substances that normally constitute muscle.

The object of this paper, therefore, is to present conclusive morphologic evidence of the pathologic changes produced in nerve, nerve ending, neurosome, and muscle by disuse, in comparison with their normal structure associated with normal use.

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## Materials and Methods

Under aseptic surgical technic, the Achilles tendon of the right gastrocnemius muscle was completely severed by transverse section from the calcaneus, and 3 mm. of the distal end of the tendon were excised in 300 white rats (*Mus norvegicus*). The gastrocnemius muscle of the left leg was allowed to remain intact and used as a control of the effects of normal use. At 24-hour intervals the morphologic changes in the right gastrocnemius muscle were compared with the normally used muscle of the left side in each of 10 rats over a 30-day period. Segments of the control and the experimental gastrocnemius muscles and sciatic nerves were simultaneously subjected to the same histologic technics. Of 60 additional white rats, 4 were selected at 48-hour intervals following tenotomy. A ligature tied at the cut end of the muscle had at its free end suspended weights that varied from 10 to 30 gm. depending upon the size of the muscle. The living muscle *in situ* was gradually restretched, prior to excision for gold impregnation, for periods of 10 to 15 minutes, alternating with 5 minutes of rest, for 3 to 5 hours.

The right hind legs of 60 other rats were completely encased in plaster of Paris casts from the toes to and including the hip joints. The cast of the hind leg was continuous with a partial body cast for stability and anchorage. The left hind leg was free for walking. Four of these rats were selected at 48-hour intervals after the application of the cast. The structural changes in the right immobilized gastrocnemius muscle were compared with those in the normally used left muscle. Comparison was made likewise with the changes at the same periods of time following disuse by tenotomy and by the cast.

The method of gold impregnation and teasing of whole muscle fibers, previously described,[1] was found superior to any other neurologic technic for the detection of the structural changes of the nerve endings in muscle. This method was checked against the current popular ones in which silver or methylene blue is used. The muscles were likewise stained with osmic acid, sudan III, sudan black, and scharlach R, as well as with ordinary stains such as hematoxylin and eosin, after fixation with formalin, Zenker's fluid, or other fixatives.

The variable structure of the motor end-plates produced by gold (Figs. 1 and 2) and methylene blue (Figs. 3 and 4) in teased fibers of the same gastrocnemius muscle of the pseudo-chameleon (*Anolis caroliniensis*) was contrasted with that produced by silver (Figs. 5, 6, and 7) in longitudinal frozen sections after formalin fixation. The chameleon was used in this experimental study because the nerve endings are two to four times larger than those found in any mammalian muscle

and the various structures may be readily compared. These three neurologic technics produced the same changes in mammalian nerve terminals as they did in those of the chameleon muscle. The revelation of the true microscopic structure of the nerve ending, therefore, is dependent upon the histologic technic employed. The granules of the sole plate of Kühne are more clearly demonstrated by the gold method than by either methylene blue or silver.

The nerve terminals are constricted by the silver method. The neurofibrillae and nuclei are more clearly revealed by this method than by any other. The gold method, followed by teasing of the whole muscle fiber, was, however, superior to any other neurologic technic for the demonstration of the normal and pathologic discharge of neurogenic substances from nerve endings into muscle. When there was a copious discharge of neurogenic substances from nerve endings that had a large diameter demonstrated by the gold method, similar portions of the same muscle subjected to the silver method revealed clear-cut neurofibrillae in the terminal axons and a periterminal network of Boeke between these axons and the anisotropic substance of the muscle. This periterminal network, therefore, was not a constant finding in all nerve endings. The silver method revealed loops, rings, terminal brushes, and terminal networks in a variable manner in the nerve endings. These were related to the variable structure of the nerve endings demonstrated by the gold technic. The conclusive evidence, therefore, that the motor nerve endings were microscopic glands of internal secretion was better demonstrated by the gold method than by any other neurologic technic.

The left and right sciatic nerves were stained with gold, silver, methylene blue, osmic acid, and various other dyes, to demonstrate myelin degeneration. Nerves and muscles were prepared in whole units and in longitudinal and cross sections. The great distortion, shrinkage, and chemical changes produced by the formalin fixation and alcoholic dehydration used in the silver method were eliminated by the use of the gold method in the study of nerve endings in muscle. The gold chloride reacted directly with the neurogenic axonic substances in nerve and muscle without need of the prior use of formalin fixation.

Formalin produced chemical changes and altered critical features of the structure of the neuromuscular apparatus. No alcoholic dehydrating agent was used in the gold method of teased whole mounts. There was some swelling of the nerve and muscle when they were placed in the citric acid or in lemon juice prior to gold impregnation, but this tended to reveal more clearly than otherwise the real nature of the union of nerve and muscle. It was realized, however, that any artificial

technic did not demonstrate, absolutely, the reality of the living structural union of nerve and muscle. The acid, however, may have acted by stabilizing the products of neurogenic secretion of nerve and muscle before they were either altered or destroyed by the chemical changes produced by the ordinary fixatives used in histologic study.

The teasing of whole muscle fibers was a better technic than cutting muscle into sections for observation of the whole neuromuscular apparatus and the anatomic relationships and changes of the epilemmal axon, hypolemmal axon, granules of Kühne, cross striations, and the granular and agranular muscle fibers. The failure, in the past, to detect morphologic changes in the nerve endings has been due to the popular choice of certain histologic methods which profoundly alter the structure of the union of nerve and muscle. In the living, this union is transparent, and up to the present time it has defied clear-cut observation, but this problem is still under investigation. The following experimental observations will be confined to the structure of nerve endings of the gastrocnemius muscle in the white rat revealed by the gold technic.

## Results

### *Effects of Normal Use on the Motor End-Plates in Dark and Light Voluntary Muscle Fibers*

The normal muscle observed after gold impregnation in teased whole muscle fibers had relatively narrow, dark, and granular muscle fibers (Fig. 8) scattered among others of various diameters which were light and relatively agranular. The granular fibers had coarse, medium-sized, and fine granules. The muscle fibers were classified as hyperchrysophilous, orthochrysophilous, hypochrysophilous, and achrysophilous based upon the intensity of the reaction of the granules with gold. There were multiple gradations of affinity of the granules with gold between the extremes. The nerve endings were usually expanded (Fig. 8) and had a decreased affinity for gold in the light fibers; the endings were usually retracted in the dark fibers.

The granules of Kühne usually formed a dense rim around the retracted nerve endings, whereas there was a quantitative diminution of these granules, to the point of complete depletion, around the extended branches of the expanded nerve endings produced by neuroprotoplasmic streaming. The light halo-like space found between the terminal axons and the granules of Kühne (Figs. 1, 2, and 30) in some normal nerve endings was not a constant finding in all motor end-plates. The evidence suggested that it may represent the interval in which submicroscopic neurogenic particles were discharged and quickly became visible by enlargement through interaction with the colloidal

granules of the muscle substance. This halo-like space is not an artifact but was absent when there was massive transmission of the irritable neurogenic substance into the sarcoplasm of the muscle fiber (Figs. 39 to 62). In a differential count of 5,000 normal nerve endings, the retracted endings varied in length from 20 to 40 $\mu$ and the expanded endings from 40 to 60 $\mu$.

The normal muscle in cross section (Figs. 13, 14, and 15) had fibers with coarse, medium-sized, and fine granules in the narrow and medium-sized muscle fibers. The extremely wide fibers had the gold-impregnated material arranged in a reticulum, or there was a marked diminution of the substance with an affinity for gold in the light, agranular fibers. There were variations, however, in the size of the granules in fibers of different diameters. The axon, nerve endings, granules of Kühne, and granules in the muscle fiber all had the same reaction to gold. The size and distribution of the granules were assumed to be related to the different degrees in the process of hydrolysis after the neurosomes were discharged into the muscle.

*Effect of Tenotomy on the Dark, Granular Type of Muscle Fiber*

During the first 2 to 3 days following tenotomy (Fig. 9), there was frequently found in some muscles a quantitative increase in the frequency of distribution of the dark muscle fibers. Between the 3rd day (Fig. 9) and the 30th day (Figs. 12 and 19) there was a gradual loss of the dark muscle fibers following tenotomy. There was, likewise, a great depletion (Fig. 12) of the nerve supply as well as of the substance of the muscle fiber by the 30th day. In some places, the hypolemmal axons of the nerve endings were absent (Fig. 12) and all that remained 30 to 50 days after tenotomy were clumps of sole plate nuclei. The structural expression of the dark, coarsely granular muscle fiber (Figs. 8, 9, and 13) was determined, therefore, by the presence of the attached and normally functioning muscle with its active innervation. It required a considerable period of time, following disuse by tenotomy, to deplete the muscle fiber of the neurogenic substance normally discharged into the muscle. Whether or not this neurogenic substance discharged into muscle may go through a certain number of cycles of breakdown and resynthesis before it is completely used up, is still undetermined by this experimental study. This neurogenic substance with a variable affinity for gold is found in the sarcoplasm. When it forms a well defined network (Figs. 20, 21, and 22), it corresponds to the sarcoplasmic boundaries of the so-called areas of Cohnheim observed in ordinary histologic preparations after formalin and Zenker's fixation.

From the 2nd to the 15th day (Figs. 25 to 27) following tenotomy the intramuscular capillaries and small veins became clearly visible due to the passive hyperemia associated with disuse.

### *Effects of Tenotomy on the Discharge of Pathologic Neurosomes from Motor End-Plates*

The nerve endings became uniformly and deeply impregnated with gold, and fusiform in shape, between the 4th and 20th days following tenotomy in the rat. There was individual variation in different animals as well as in different fibers of the gastrocnemius muscle in the same animal in the appearance of these morphologic changes (Figs. 23 to 27). The molding of the nerve endings into various shapes, such as oval and fusiform, was produced by the progressive shrinkage of the muscle fiber due to the loss of muscle substance (Figs. 23 to 27, and 40 to 62). The course of the transformation of the motor end-plates into either giant globular or giant fusiform neurosomes may be traced by arranging in sequence a selection from a large number of motor end-plates studied under the microscope (Figs. 40 to 62). The evidence is conclusive that a large mass of material found in the giant neurosomes is contributed by the motor end-plates even though there may be an interaction of this neurogenic material with sarcoplasmic granules in the muscle substance.

During the early period following tenotomy (Figs. 40 to 46), the motor end-plates were uniformly impregnated with gold with little or no evidence of distinct hypolemmal axons. This interaction with gold was due to the presence in the nerve ending of an increased quantity of material with an affinity for gold. The normal and abnormal experimental muscles were run through the various stages of the technic simultaneously. Frequent comparisons of the normal reaction to gold (Figs. 30 to 32, and 35 to 38) were made with the abnormal reactions of the motor end-plates (Figs. 28, 29, 33, 34, 39, and 40 to 62). The pathologic changes following tenotomy were proved to be due, therefore, not to a faulty technic or to overimpregnation with gold, but to the intrinsic changes produced in the neuromuscular apparatus following tenotomy.

On the 10th to the 15th days following tenotomy (Figs. 10 and 11) there was a discharge of either small or large gold-impregnated neurosomes from the hyperchrysophilous motor end-plates. In some places the epilemmal axons manifested a decrease in the quantity of material with an affinity for gold (Figs. 10 and 11) in comparison with both the normal innervation and the innervation 48 hours following tenotomy (Figs. 8 and 9). There was structural manifestation of various stages in the genesis and discharge of giant globular and fusiform

neurosomes from the oblong and spindle-shaped nerve endings which were hyperchrysophilous (Figs. 10 and 11, and 40 to 62).

From the 3rd to the 15th days following tenotomy, in certain places in the muscle a progressive increase in the number of giant neurosomes was found. From the 15th to the 30th days following tenotomy, there was a progressive decrease in their number. The discharged giant neurosomes were irregularly scattered in the myoplasm. Some of them were uniformly and deeply impregnated with gold. Some were light in the center and dark at their tapering ends. Others were light at the ends and dark in the center. Still others were very faintly impregnated with gold, and their contained granules were arranged in cross striations undergoing progressive alignment with the cross striations of the muscle fiber.

These fusiform neurosomes varied from 10 to 275 $\mu$ in length and from 4 to 60 $\mu$ through the widest transverse diameter. In some places there were small, oblong and fusiform neurosomes, similar in structure and staining reaction to gold to those observed during the early stages after nerve section in denervated muscle.[1] The large fusiform neurosomes were produced by a streamlining effect (Figs. 63 to 75) of the cross-striated myoplasm during the process of migration of the neurosomes from the nerve ending and dispersion into the protoplasm of the muscle fiber. Some of these neurosomes in transverse sections were immediately under the sarcolemma whereas others occupied the center (Figs. 16 to 18, and 20 to 22) of the muscle fibers. During disuse atrophy there was a progressive loss of the normal dark muscle fibers and of the normal coarse granules (Figs. 16 to 18, and 20 to 22) of neurosomes. The granules were either very fine and dispersed, or aggregated into the giant fusiform neurosomes. In cross sections (Figs. 16 to 18, and 20 to 22) the giant neurosomes were either uniformly deeply impregnated with gold, or they had a light center, or a rim of granules faintly reacting to gold.

This morphologic evidence strongly suggested that the visualization by photography of the pathologic nerve impulse was determined by the accumulation of neurogenic substances produced by a retardation in the rate of discharge, diffusion, and dissolution by hydrolysis of the neurogenic secretion from the motor nerve ending. This neurogenic secretion interacted with the muscle substance. The friction of the myogenic fluid slowed down the transmission of the giant neurosomes. The resisting drag of the viscous myoplasm on the migrating neurosomes was associated with the formation of whirlpools or eddies. The resulting eddies or vortices were detected by the turbulence of the related cross striations at the prow and in the wake of the giant

neurosomes. The streamlining of the neurosomes resulted in tapering ends. The pointed rear end is more important in streamlining than the front end. In some locations (Fig. 65) the neurosomes assumed the "teardrop design" of streamlining with one end blunt and convex like that of a torpedo. This design was modified, however, by the fact that the neurosomes are not inert particles moving in the muscle protoplasm but are subjected to colloidal chemical action and reaction with the muscle substance. Progressive changes in the hydrolysis of the giant neurosomes were clearly evident (Figs. 63 to 75).

### *Effects of Disuse from Casts on the Production of Pathologic Neurosomes*

The effects produced on the nerve endings of the gastrocnemius muscle by immobolizing in a plaster cast the right hind leg of a rat were essentially similar to those found after disuse by tenotomy. The deformations of the nerve endings after disuse produced by the casts occurred later than those demonstrated after tenotomy. This may be associated with the slower rate of muscle atrophy after immobilization by a cast than that produced following tenotomy. The neurosomes discharged from nerve endings during the early stages after the application of the cast were usually more nearly spherical than those generated by tenotomy (Figs. 47 to 56).

The neurosomes in tenotomized muscle were generally elongated and fusiform (Figs. 57 to 75). It is assumed that the configuration of the neurosomes was determined by the rate of atrophy and the physical changes of viscosity of the myoplasm produced by disuse, as well as by a slowing down in the rate of secretion, diffusion, and hydrolytic dissolution of the neurosomes.

### *Effects of Tenotomy on the Nerve Supply of the Gastrocnemius Muscle*

During the first 10 days after the right gastrocnemius muscle was tenotomized there was an increase in the weight of the nerve fibers supplying the muscle. Corresponding lengths of 5 mm. of the control left and experimental right nerves, from their entrances into the muscles, were excised and weighed in 30 rats. There was an increase in the weight of the nerve fibers of the right experimental side that varied from 10 to 40 per cent over that of the left control nerve fibers. This increase was due to: (1) edema associated with the passive hyperemia of the intraneural blood vessels; (2) increase in diameter of the axis cylinder; and (3) accumulation of lipoidal material due to demyelinization of the medullated nerves. These pathologic products of nerve dystrophy following disuse augmented the size of the axis cylinder

(Figs. 28, 29, 33, and 34) and were then discharged periodically in a centrifugal direction into the dystrophic muscle (Figs. 39 to 75).

In frozen sections after formalin fixation and silver impregnation the enlarged epilemmal and hypolemmal axons during the first week after either tenotomy or the application of a cast showed an increased number of neurofibrillae. This increase was associated with a dilatation and increase in quantity of neurogenic material in the axon when studied with gold (Figs. 28, 29, 33, 34, and 39 to 46). There was an apparent increase in number of the nuclei in the sole of some nerve endings observed by the silver method following atrophy of disuse. This may have been an aggregation by migration of neighboring nuclei in the muscle and not an absolute increase in total number of nuclei in the whole muscle fiber.

### *Effect of Restretching the Tenotomized Muscle*

The re-establishment of partial muscle stretch in some fibers of the muscle by the regenerative attachment of the tendon to the subcutaneous tissue is a variable which we took into consideration in evaluating our results. In some of the rats in our series, the tendon of the muscle was freshly cut at 7-day intervals to eliminate the effects of the partial re-establishment of stretch by union of tendon to subcutaneous tissue. The experimental evidence strongly suggested that the loss of normal muscle stretch determined the retardation in the rate of discharge, diffusion, and disappearance by hydrolysis of the giant neurosomes. This conception was supported by the great decrease in the number, and in some muscles the total absence, of the giant neurosomes in the series of 60 living muscles experimentally restretched *in situ* before excision and gold impregnation. Four muscles were restretched at 48-hour intervals until the 30th day after tenotomy. The living muscles were restretched for periods of 10 to 15 minutes alternating with 5 minutes of rest, for 3 to 5 hours. The muscles were restretched by a ligature tied at the distal cut end of the muscle, from which weights were suspended that varied from 10 to 30 gm. depending upon the size of the muscle.

### *Effect of Tenotomy on Muscle Weight*

The gastrocnemius muscles lost 30 to 50 per cent of their weight, compared with the normal, during the first month after tenotomy. There were individual variations in the rate of atrophy and in the morphologic changes of the neuromuscular apparatus and fibers of the gastrocnemius muscles. These variations were found not only in muscles from different animals examined after the same time interval following tenotomy, but also in different fibers in the same muscle. It

was necessary, therefore, to survey great numbers of muscle fibers to detect the average trend of the morphologic changes.

## Discussion

The disuse of muscle by tenotomy and from a cast produces dystrophy or a nutritional disorder of nerve and muscle. Langley [2] recognized the analogy between denervated and fatigued muscle. Others have noted the resemblance between the atrophy of muscle due to loss of nerve supply and that due to extreme starvation.

What is the underlying mechanism of muscle atrophy? The answer to this question involves our conception of the structure of muscle tissue. Langley,[3] upon the basis of chemical experiments, identified two constituents: (1) the irritable substance in the sarcoplasm, and (2) the contractile molecule in the myofibrillae. Tower [4] has defended, likewise, the proposition that sarcoplasm and myofibrils are discrete. The possibility that these constituents of the muscle cytoplasm form a continuum has been considered also by Tower. Hürthle and Wachholder,[5] however, concluded that the sarcoplasm is merely a schematic abstraction of the histologic structure of muscle.

Langley [3] mentioned two possibilities regarding the localization of special excitable substances in muscle cells. He found that curare decreases the excitability of the muscle to the nicotine stimulus and assumed that it decreases also the irritability of the muscle to stimuli arriving by the nerves. He assumed that the compounds which these poisons form with the muscle are less irritable and conductive than the normal muscle substance. Since neither curare nor nicotine, even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curare is not identical with the substance which contracts. Langley called this specially excitable constituent the receptive substance. It receives the stimulus and, by transmitting it, causes contraction. He stated that if nicotine causes contraction of the fibrillae and not of the sarcoplasm, there are two possibilities: the receptive substance may be part of the sarcoplasm, or it may be a radical of the contractile molecule. Langley clearly stated that his hypothesis of a specific excitable substance in muscle required that the nerve impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve, a theory suggested in the first instance by du Bois-Reymond.[6] This theory was placed on a substantial basis by Loewi,[7] Dale, Feldberg, and Vogt,[8] and others. Langley considered the problem from a theoretical point of view, regarding the localization of the special receptive substance as either in the immediate neighborhood of the nerve ending or dissemi-

nated throughout the length of the muscle fiber. He concluded from his experiments that it is the receptive substance in the muscle cell, rather than the nerve endings, which is stimulated or paralyzed by curare and nicotine. The receptive substance was also involved in some change during fatigue, according to Langley's conception.

Denervation atrophy abolishes the morphologic differences between the types of muscle fibers. Knoll and Hauer [9] observed that the thick fibers decreased in size much faster than the thin ones and that after 35 days there was no longer any distinction among the fiber types in the atrophied muscle. The granules which appeared when the normal thin muscle fibers were excised and observed fresh, disappeared after denervation. The appearance of these granules in the normally thin fibers, therefore, was associated in some way with the normally functioning innervation.

The atrophy of disuse of muscle following tenotomy or the application of a cast likewise abolishes the morphologic differences between the types of muscle fibers. There was a rapid diminution in size of the thick fibers and a progressive loss of dispersion of the characteristic dark gold-impregnated granules in the thin muscle fibers. During the early period following tenotomy of innervated muscle there is a massive aggregation of the granules into coagulated clumps that vary in size. Disuse slows down the rate of the nerve impulse and forms large viscous droplets discharged and delayed in dissolution from the nerve ending. The pathologic neurogenic substance associated with the abnormal nerve impulse retarded in rate of discharge from the dystrophic nerve ending is morphologically detected by this method of retardation. Inactivity abolishes, therefore, the normal agitation of dispersion of the neurogenic granules and reticulum in intimate relationship with the myofibrillae.

This clumping of neurogenic substances in the sarcoplasm, by disuse of the muscle, may be considered an intramuscular method of denervation of the myofibrillae inside of the muscle fiber. The efficiency of normal innervation appeared to depend upon the normal activity of diffusion of neurogenic granules that undergo progressive decrease in size by hydrolysis and thereby make intimate contact with the colloidal myofibrillae. The electric signs of the normal nerve impulse are assumed to be associated with the substantial transfer of the tenuous and evanescent neurogenic granules which excite the myofibrils to the rapid type of contraction and which likewise underlie the normal tonus of muscle.

The basic mechanism in the physiologic processes of use, increased use by exercise, and disuse, resulting, respectively, in muscle maintenance of health, in hypertrophy, and in atrophy of disuse and disease, is

unknown. Young [10] stated that "the theories of the causes and nature of muscular atrophy are numerous but none is conclusive." This conclusion is held likewise by Carlson and Johnson.[11] The parts of the living body increase and decrease in size in proportion to the functional demand or use, but the essential underlying cause of these changes has remained elusive. The changes in physiologic structure during use and disuse of the neuromuscular apparatus have remained an unexplored field in spite of our present information that loss of motor innervation results in the following characteristic changes: (1) the quantitative decrease of the substance in the muscle fiber, and (2) the hyperexcitability of the skeletal fibers to mechanical and chemical (acetylcholine) stimuli. The hyperexcitability manifest by fibrillations may be prevented by quinidine, but Solandt and Magladery [12] observed that the denervated muscle continues to shrink. The slow, incoordinate activity of fibrillation, therefore, appears not to be the cause of muscular atrophy. It has been demonstrated by Gutmann and Gutmann,[13] Hines,[14] Eccles,[15] and Solandt, DeLury, and Hunter [16] that the volume of denervated muscle may be fairly well maintained for a certain period by appropriate electrical exercises. Evidently, periodic optimum tension of traction and contraction (work) of muscles anchored to their attachments is necessary for muscle maintenance during health. Suggestive evidence was demonstrated previously that an optimum periodic tension of differential growth is necessary for the genesis of both smooth and skeletal muscle.[17] Evidence was presented also that anatomic and experimental dampers to the lateral expansion of the stretched muscle fibers produce a replacement of muscle by fibrous tissue.[18]

The normal tension, or stretch, and work of the intact muscle attached to origin and insertion appear to be necessary for the normal rate of discharge of granules from the motor end-plates. Abundant evidence has been produced which appears to support the statement that under normal conditions there is a cloud of very fine, evanescent granules discharged rhythmically into the muscle from the motor nerve endings. This secretion is periodic and under normal conditions the rate of diffusion and disappearance of the granules by hydrolysis is very rapid. Evidence has been presented which suggests that there is a close relationship between the granular and agranular muscle fibers and the rapid cycle of discharge, diffusion, and disappearance of the neurogenic granules in muscle. When the rate of discharge and hydrolysis of the neurogenic secretion has been retarded by disuse, there is a correlated change in the morphologic expression of the discharge from the motor nerve endings.

This secretory process from the nerve endings may be slowed down by disuse of the innervated gastrocnemius muscle following tenotomy. By tenotomy, the normal stretch or tension of the muscle is destroyed. The lax muscle fibers released from one attachment are analogous to the broken strings of a violin. The detached strings are incapable of normal vibratory response because of loss of tone or tune. The rate of discharge of neurogenic substance into the muscle appears to depend upon the reciprocal interaction between nerve and muscle. The normal mechanical tension of the attached muscle fibers appears to determine the normal periodical flow of neurogenic substances into the muscle. The normally attached muscle fiber appears to act like an alternate pressure and suction chamber upon the nerve ending. This nerve ending appears to be a biologic jet valve or ejector of the neurogenic secretion. Under normal conditions the rate of discharge, diffusion, and disappearance is excessively rapid. Under conditions of disuse this rate is greatly retarded. There is a characteristic structural expression reflected by the atrophy of disuse of the neuromuscular apparatus following tenotomy. The release of normal muscle stretch by tenotomy decreases the demand for, and therefore the rate of, neurogenic discharge into the abnormally flaccid muscle fibers.

This laxity of the muscle fibers slows down the normal rate and permits accumulation of the discharge of the neurogenic secretion. The longitudinally striated myoplasm is composed of (1) the myofibrils which vary in width and in distinctness, and (2) the sarcoplasm containing the neurogenic substance which, likewise, varies in size from large, pathologic neurosomes to the very small, physiologic neurosomes. Some of the muscle fibers are agranular. The granular and agranular muscle fibers may, therefore, be designated, respectively, as the neurosomic and aneurosomic fibers. These granules are large in the narrow, dark, granular muscle fiber. The so-called sarcoplasm contains these neurosomes.

The normally discharged neurosomes react in a variable manner to gold, thionin, and lipoidal stains. Evidence now at hand supports the statement that the problems of fatty metamorphosis, Zenker's hyaline degeneration, and the pathogenesis of dystrophy of muscle are closely related to aberrations of the neuromuscular apparatus and alterations in the secretion of neurosomes into muscle. In the past, many of the fine physiologic neurosomes have been identified variously as the interstitial granules of Kölliker,[19] the J and Q granules of Holmgren,[20] and the nutritional liposomes of Albrecht,[21] Bell,[22] and Denny-Brown.[23]

In some of the light, agranular fibers there is a fine network of neurogenic material in the sarcoplasm, and in its meshes are found the cross

sections of single or multiple myofibrils. This neurogenic network, in some places, surrounds each myofibril, whereas in other locations in the fibers of the same muscle this fine network is absent. When irregular groups of myofibrils are surrounded by the coarse network of neurogenic material, the so-called areas of Cohnheim are formed in the cross section of the muscle fiber. There is a high degree of variability in the presence and absence of areas of Cohnheim. These Cohnheim areas are not constant, fixed, structural arrangements of the myofibrils in each muscle fiber, as is now assumed. The problem of muscle structure requires a complete restudy based on experimental, controlled conditions.

The evidence presented in this paper supports the principle of the *double dependence* of nerve and muscle proposed by Young.[10] He stated that muscle receives its stimulation from nerve and exercises constraint against other muscles or outside forces. Muscle will atrophy if given too little direction from above, as after total denervation or isolation of lower from upper neurones; it will also atrophy if it is left relaxed and, therefore, cannot contract against resistance. However, until the structure of nerve and muscle is more adequately understood than it is at the present time, the problem of muscle maintenance, hypertrophy, and atrophy will remain unsolved. The study of the after-effects of infantile paralysis and other primary and secondary diseases and dystrophies of nerve and muscle will not be clarified until the real structure of nerve and muscle under physiologic and pathologic conditions is known.

The experimental evidence presented in this paper substantiates the proposition that one important factor underlying the mechanism of muscle atrophy of disuse is the substantial loss of the normal neurosomes discharged from nerve to muscle. The loss or decrease in the amount of this normal morphologic transmitter associated with the electric signs of the normal nerve impulse parallels the decrease in the mass of the myoplasm following inactivity of muscle.

## Summary

Experimental evidence has been presented which supports the following statements:

1. The apparent structure of the nerve ending in the skeletal muscle fiber is dependent upon the specific histologic technic employed. The gold method, followed by the teasing of whole muscle fibers, is better than other technics employing silver or methylene blue for demonstrating the normal and pathologic neurosomes discharged from the motor nerve ending into the muscle fiber.

2. The visualization by photography of the morphologic transmis-

sion of an increased mass of substance from nerve to muscle, associated with the pathologic nerve impulses, was determined by a slowing down of the rate of nervous action by inactivity of the associated muscle.

3. During the early stages of disuse atrophy of rat muscle produced by tenotomy or by the application of a cast, the neurogenic secretion is retarded in the rate of discharge, diffusion, and dissolution by hydrolysis. The enlarged droplets of nerve substance in muscle may be observed in different stages of solution and photographed for detailed study. The normal rate of discharge, diffusion, and dissolution is so rapid that detailed microscopic study of the physiologic neurosomes is difficult.

4. There is a gradual loss of the dark, granular muscle fibers during the first month after tenotomy, as well as a rapid decrease in mass of the light, thick muscle fibers. The physiologic production of the granular fibers appears to be dependent upon the normal function of the innervation which discharges normal neurosomes into the muscle fiber. The evidence suggests that the coarse, medium-sized, or fine granules and the gold-impregnated network in the sarcoplasm are different stages in the hydrolysis, diffusion, and intimate commingling of the irritable nerve substance in the colloidal and fibrillar muscle substance. The inconstant structure of the areas of Cohnheim appears to correspond with the phase of the gold-impregnated network formed by the progressive hydrolysis of the neurosomes in the sarcoplasm.

5. The pliability of the active muscle appears to depend upon the normal colloidal compounding of the nerve and muscle substances. The muscle stiffness associated with disuse is accompanied by an abnormal aggregation of the nerve substance into large clumps inside the muscle fiber, resulting in a loss of the normal dispersion of fine granules of nerve substance. This abnormal clumping of nerve substance in the inactive muscle fiber appears to be a form of partial intramuscular denervation. The normal dispersion of fine neurogenic particles in muscle is dependent upon the normal reciprocal activity of nerve and muscle. Inactivity slows down the periodic neurogenic discharge of granules into muscle and appears to increase the viscosity and delay the disappearance of the pathologic nerve fluid flowing into muscle.

6. The giant fusiform neurosomes that appear during the early period following tenotomy disappear or are greatly reduced in number when the living muscle *in situ* is restretched adequately prior to excision and gold impregnation.

7. The increase in size and weight of the dystrophic nerve fibers supplying the gastrocnemius muscle is associated with the following structural changes: (1) edema and passive hyperemia of the intra-

neural blood vessels; (2) increase in diameter of the axons; (3) progressive demyelinization, the products of which are discharged centrifugally through the axon into the dystrophic muscle. The evidence appears to support the conclusion that there is a continuous generation of pathologic material by the dystrophic nerve for 2 or 3 weeks following tenotomy. Over a period of time, the mass of abnormal nerve substance discharged into the dystrophic muscle would be considerably greater than the initial mass of nerve fibers at the time of tenotomy.

8. One factor in the atrophy of disuse of muscle appears, therefore, to be a substantial loss of the discharge of the normally fine neurosomes from nerve endings into skeletal muscle. This loss of physiologic neurosomes is associated with decrease in the mass of the myoplasm.

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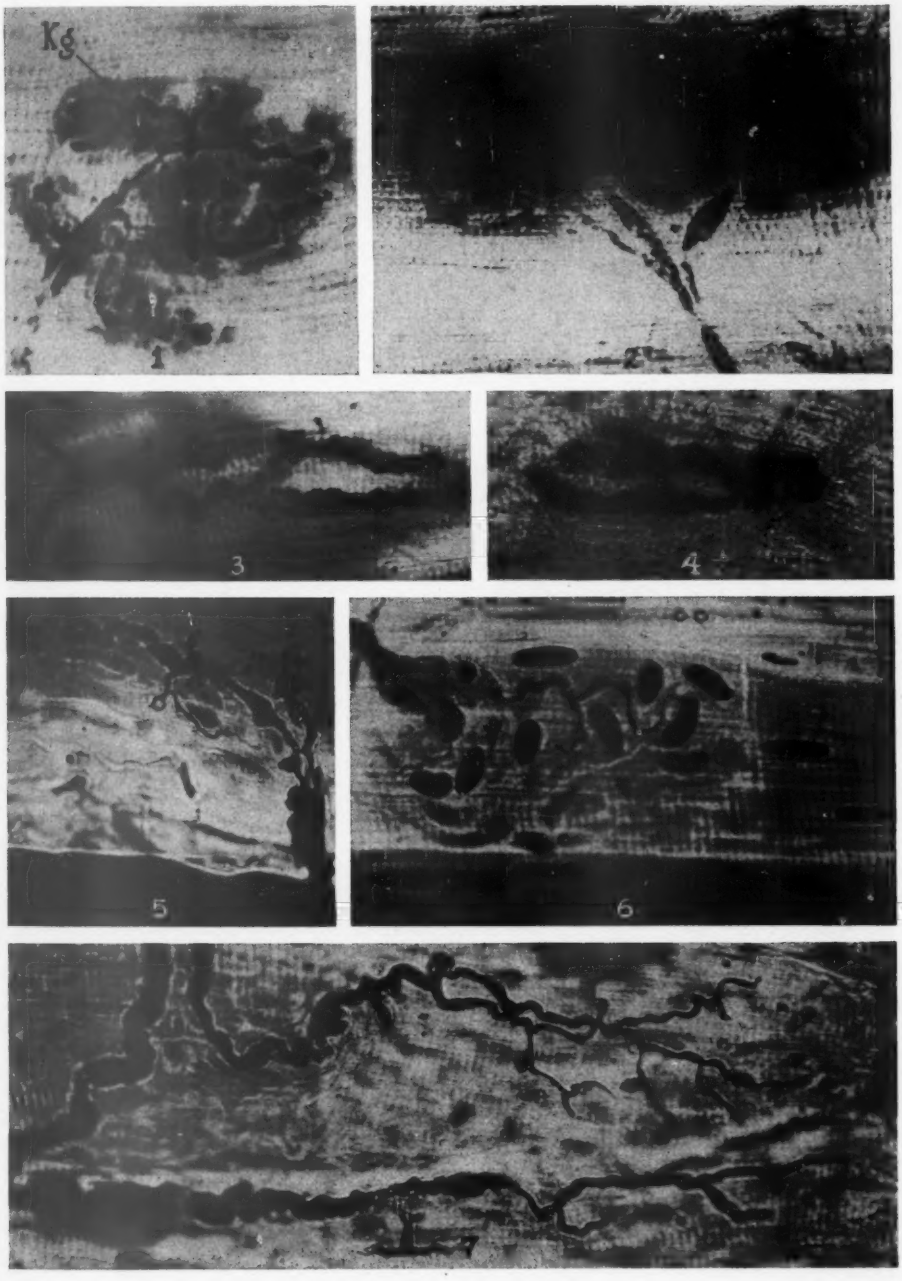
## DESCRIPTION OF PLATES

The photomicrographs of Figures 1 to 75 are from either teased whole muscle fibers (gastrocnemius muscle) and nerve endings, or from cross or longitudinal sections of muscle from the white rat (*Mus norvegicus*) and pseudo-chameleon (*Anolis caroliniensis*). The muscle was previously prepared by one of three methods, namely, gold, methylene blue, or silver. The photographs were direct contact prints from negatives exposed through the microscope and subsequently enlarged. They may be compared directly, therefore, with those previously published. In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; "NS" or "Gns," small or giant pathologic fusiform neurosomes or neurogenic particles discharged from nerve endings into the myoplasm; "Kg," Kühne's granules, or normal physiologic neurosomes; "NE," nerve endings. The negatives and prints were not retouched.

### PLATE 28

FIGS. 1 to 7. Differential structural effects of three different neurologic technics are demonstrated on the neuromuscular apparatus in the same gastrocnemius muscle of the pseudo-chameleon (*Anolis caroliniensis*), used because the motor end-plates are two to four times larger than in mammalian muscle. The variable appearances of the motor end-plates produced by gold (Figs. 1 and 2) and methylene blue (Figs. 3 and 4) in teased whole muscle fibers are contrasted with those produced by silver (Figs. 5, 6, and 7) in longitudinal sections after formalin fixation. The differences in the structure of the nerve terminals produced by these three technics are found in mammalian as well as chameleon muscle. The granules of Kühne (Kg) are clearly visible in some of the preparations made with gold impregnation (Figs. 1 and 2) but are invisible or poorly shown in those made with methylene blue (Figs. 3 and 4) and silver (Figs. 5, 6, and 7). The tremendous deformation of the nerve terminals produced by formalin fixation, silver impregnation, and alcoholic dehydration (Figs. 5, 6, and 7) is clearly apparent. No such deformation occurs when the gold (Figs. 1 and 2) or the methylene blue (Figs. 3 and 4) methods, which require no fixative, are employed. The nuclei contain nucleoli and are in close relation to the terminal filaments of the nerve endings. These nuclei are more clearly observed after silver impregnation (Figs. 5, 6, and 7). Rings, loops, terminal brushes, and networks are found on certain nerve endings after the silver method is used. The nuclei form clear oval spaces surrounded by granules after the use of gold impregnation. The technic of choice, therefore, to demonstrate the neurogenic granular discharge (Kg) into muscle from the nerve endings is gold impregnation followed by teasing of the whole muscle fibers (Figs. 1 and 2). The periterminal network of Boeke is found in silver preparations, but in place of this network a copious accumulation of the granules of Kühne is demonstrated by the gold method. When these granules are depleted or absent around the nerve terminals, the periterminal network is absent. This evidence suggests that the preparation of muscle by the silver method converts the granules of Kühne into a network. This network is found between the end of the nerve and the anisotropic substance of the muscle fiber. × 850.


Kg
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## PLATE 29

Figs. 8 to 12. Gold technic, teased whole muscle fiber. × 200.

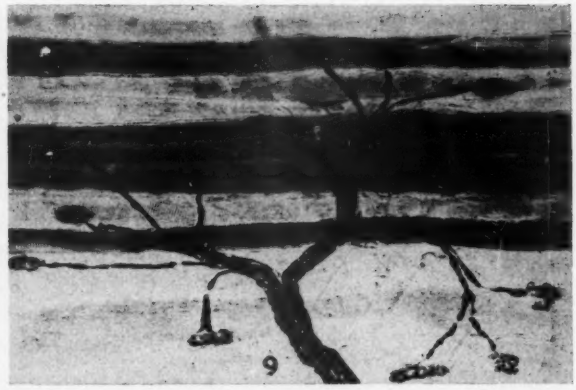
FIG. 8. Spray of medullated nerve fibers and nerve endings from a relatively normal gastrocnemius muscle removed from an etherized rat. The retracted nerve ending is in a dark, coarsely granular muscle fiber, and the expanded nerve endings are in the light, finely granular or agranular muscle fibers. The normally functioning muscle manifests fractional contraction, the structural expression of which is the differential structure of the muscle fibers and nerve endings. The dark, granular muscle fiber is related to a hyperchrysophilous nerve ending surrounded by fine granules of Kühne or physiologic neurosomes. The light, relatively agranular fibers are related to expanded nerve endings, which are in various stages of depletion of the surrounding physiologic neurosomes. The types of muscle fibers are related to the different phases in the discharge, diffusion, and dissolution by hydrolysis of normal neurosomes secreted from the nerve endings, as well as to the granules secreted by the nuclei in the sole plate and in the muscle.

FIG. 9. Spray of medullated nerve fibers and nerve endings in the gastrocnemius muscle 48 hours after tenotomy. There is an increase in number of the dark, granular muscle fibers, and in the diameter and staining capacity of the epilemmal axons.

FIG. 10. Motor innervation of the gastrocnemius muscle 15 days after tenotomy. The dark, granular muscle fibers are decreased in number. There is hyperchrysophilia of the fusiform nerve endings. There is retardation in the rate of discharge, diffusion, and dissolution by hydrolysis of the neurogenic granules, manifested by a substantial accumulation of the droplets into giant fusiform neurosomes.

FIG. 11. Motor innervation of gastrocnemius muscle 10 days after tenotomy. The dark, granular muscle fibers are decreased in number. There is hyperchrysophilia of the nerve endings and a slowing down in rate of discharge, diffusion, and dissolution, manifested by small pathologic neurosomes in the muscle. The small or giant neurosomes may appear at any time from 1 to 21 days after tenotomy.

FIG. 12. Depletion of innervation and gradual loss of dark muscle fibers in the atrophic gastrocnemius muscle 30 days after tenotomy. The dark, granular muscle fiber, therefore, is the structural expression of the normally functioning motor nerve supply that periodically discharges physiologic neurosomes into the muscle fiber.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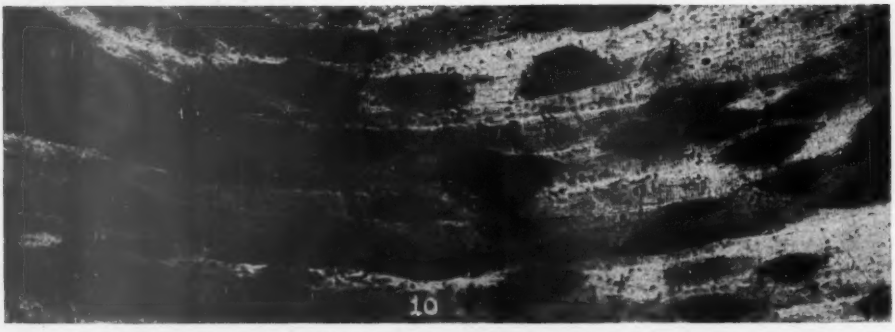
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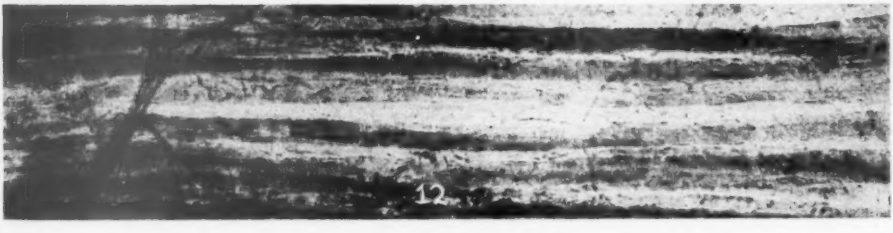
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## PLATE 30

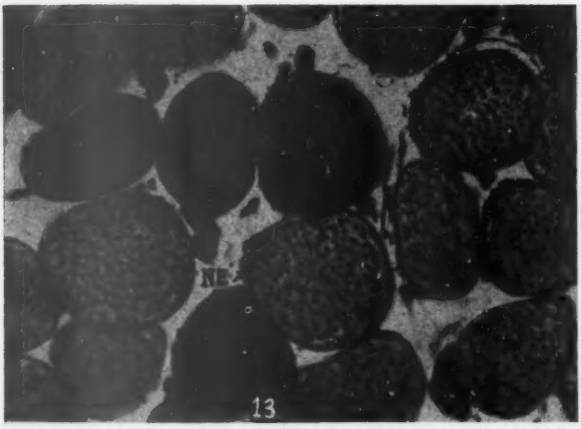
Figs. 13 to 19. Gold technic, frozen cross sections. × 400.

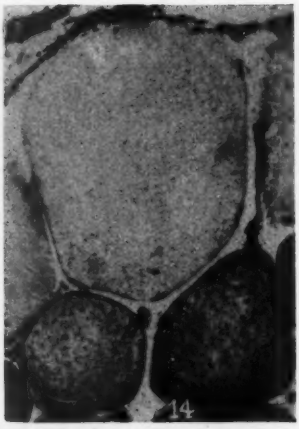
FIGS. 13, 14, and 15. Cross sections of the normal gastrocnemius muscle fibers of the rat are demonstrated. Relatively narrow, coarsely granular fibers, medium-sized and finely granular fibers, as well as wide, relatively agranular and reticulated fibers are evident. The network is clearly presented in some of the fibers (Figs. 20 to 22). This fine, chrysophilous network of the large muscle fibers, and the dissolution of this net, appear to be stages in the hydrolysis of the large granular neurosomes found in the small, dark muscle fiber. These multiform neurosomes occupy the space of the sarcoplasm found between the myofibrils and groups of myofibrils.

FIGS. 16 and 17. Cross sections of giant fusiform neurosomes (Gns), in various locations of the gastrocnemius muscle fibers 10 days after tenotomy. These pathologic neurosomes have the same affinity for gold as have the nerve endings (Fig. 17, NE). These neurosomes are either uniformly and densely impregnated with gold or they have a light center composed of fine granules and forming a circle or oval in cross section. The material of these fusiform spaces is soluble in xylol and alcohol, and in ordinary histologic preparations clear clefts in the muscle fiber are produced. The cleavage of the myoplasm described in muscle dystrophy may be produced by these neurosomes which are dissolved in ordinary preparations.

FIG. 18. Cross sections of muscle fibers 15 days after tenotomy. The small pathologic neurosomes are densely stained in a manner similar to the epilemmal and hypolemmal axons of the nerve endings. These pathologic neurosomes are at the periphery, the center, or between these two locations. There are single, double, or multiple neurosomes.

FIG. 19. There is definite atrophy of the muscle 30 days after tenotomy. The dark, granular muscle fiber (Fig. 13) is practically eliminated after 30 days of disuse atrophy.


NE
13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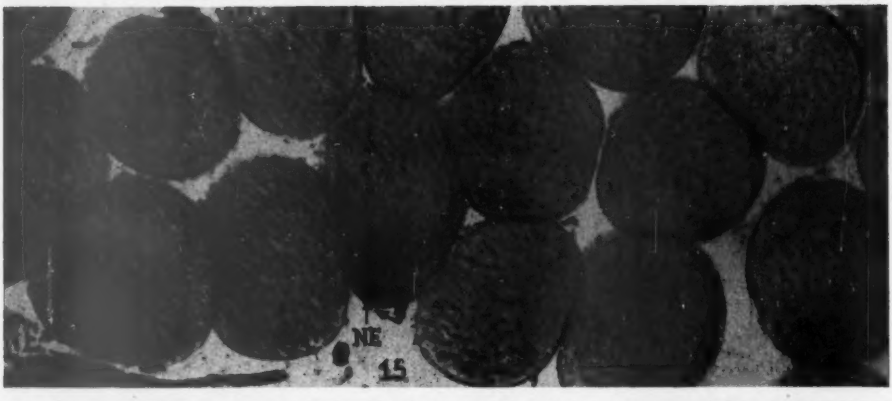
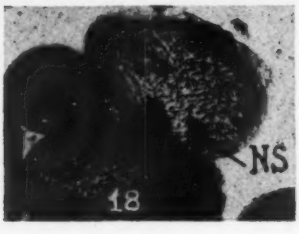

NE
15

Gns
16

NE


NS
18

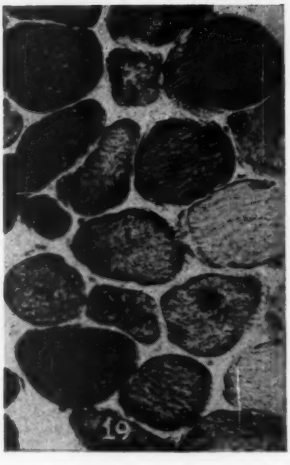
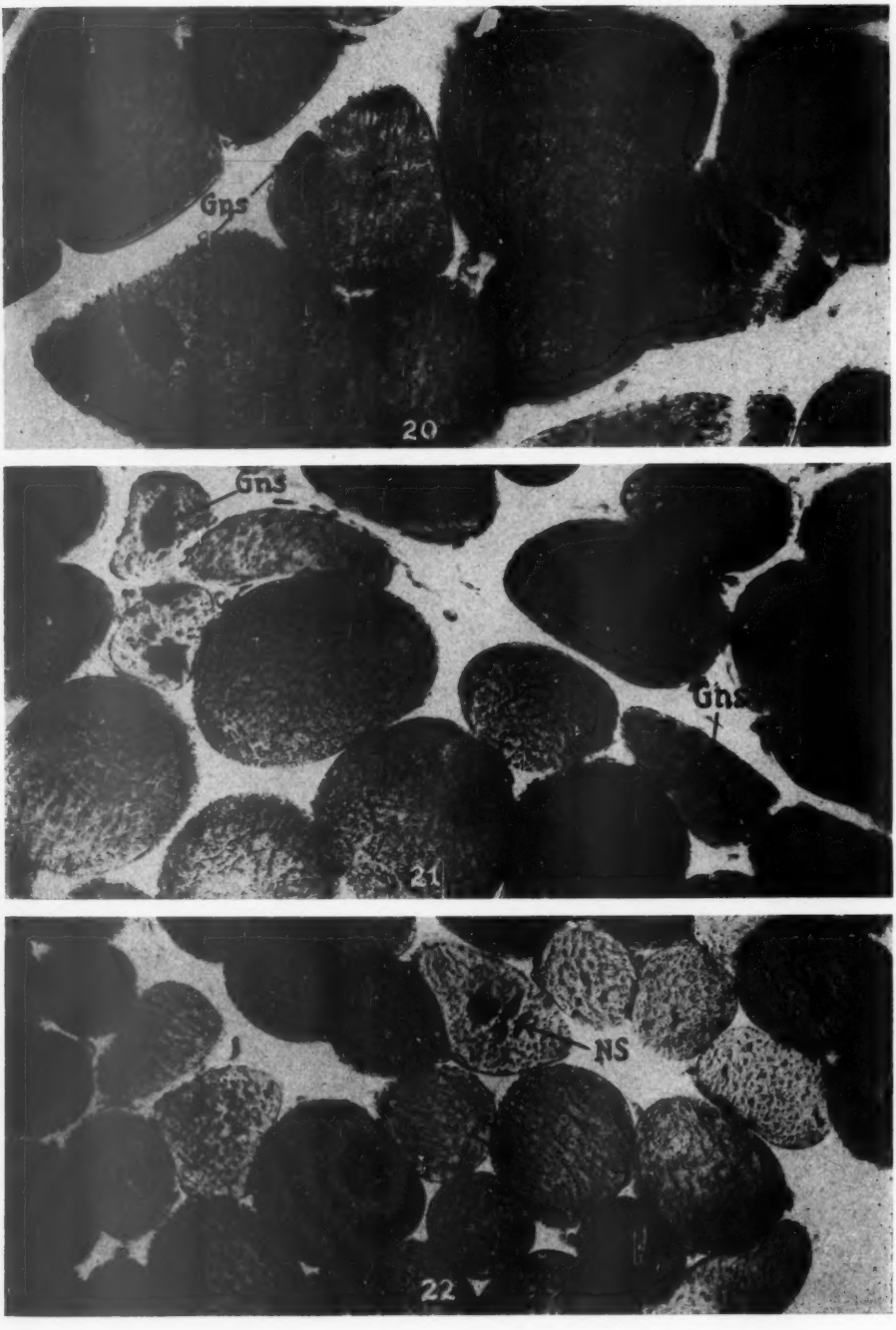

19

PLATE 31

Figs. 20 to 22. Gold technic, frozen cross sections. × 400.

FIGS. 20 and 21. Cross sections of giant fusiform neurosomes (Gns) in gastrocnemius muscle 10 days after tenotomy. In some muscle fibers the neurosomes form a large dark hub, whereas in other fibers these bodies are found at the periphery under the sarcolemma. In certain fibers (Fig. 21) the agglutination of the gold-impregnated material is found in the small, light muscle fibers undergoing considerable atrophy. In such muscle fibers there appears to be a lack of the normal diffusion of the gold-impregnated material throughout the muscle fiber. The areas of Cohnheim appear to be formed by the coarser strands forming a network of the gold-impregnated material, which has not become accumulated into the giant fusiform neurosomes.

FIG. 22. Cross sections of small neurosomes 15 days after tenotomy of the gastrocnemius muscle. In those muscle fibers in which the gold-impregnated material forms a uniform network, there is an absence of the pathologic neurosomes. Various stages in the aggregation of gold-impregnated granules are found in different muscle fibers. During atrophy of disuse by tenotomy, there is a progressive loss of the characteristic coarse and darkly granular muscle fiber observed in the normal muscle in relationship to a normal innervation (for comparison with Fig. 13).

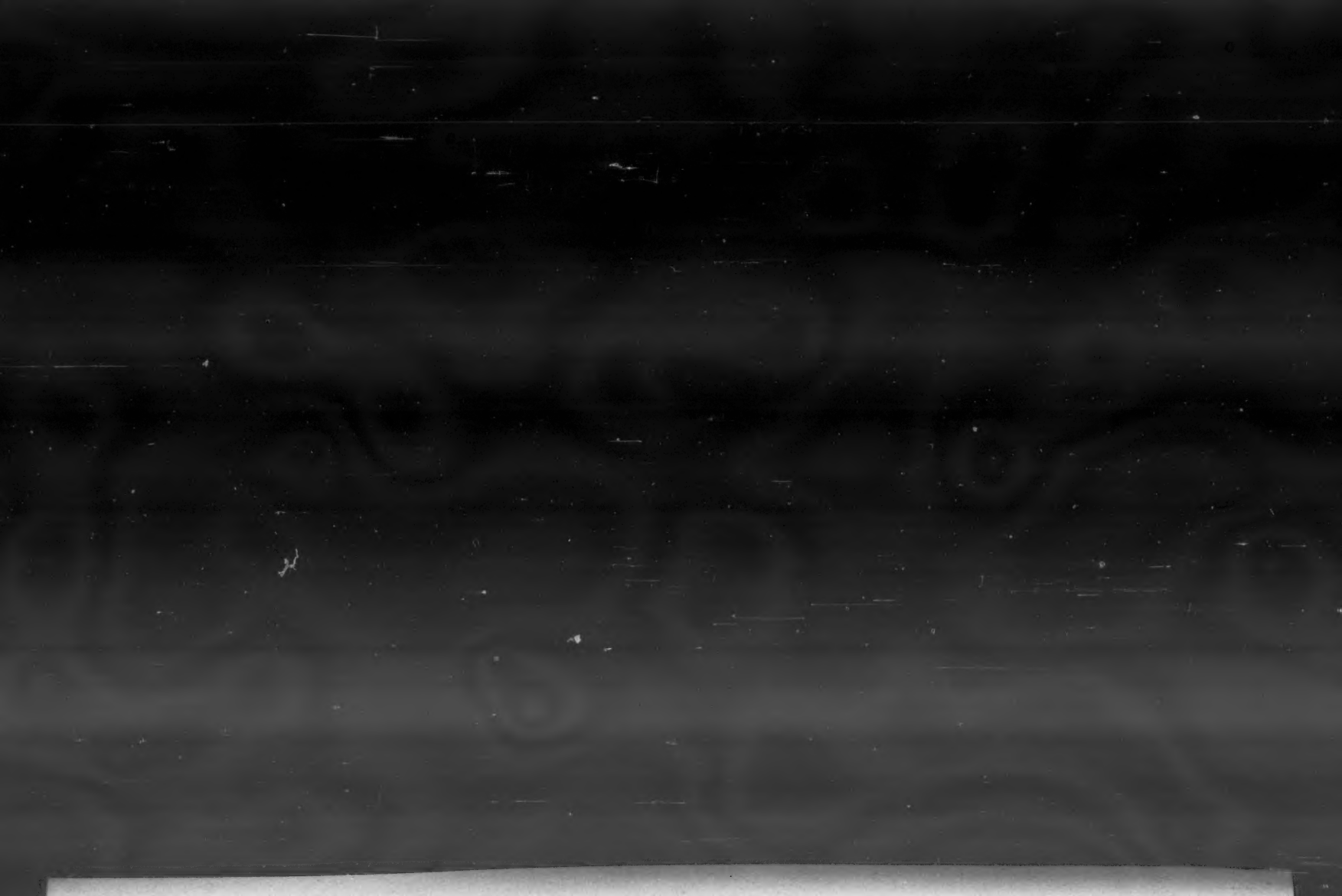

Gns
20
Gns
Gns
21
NS
22

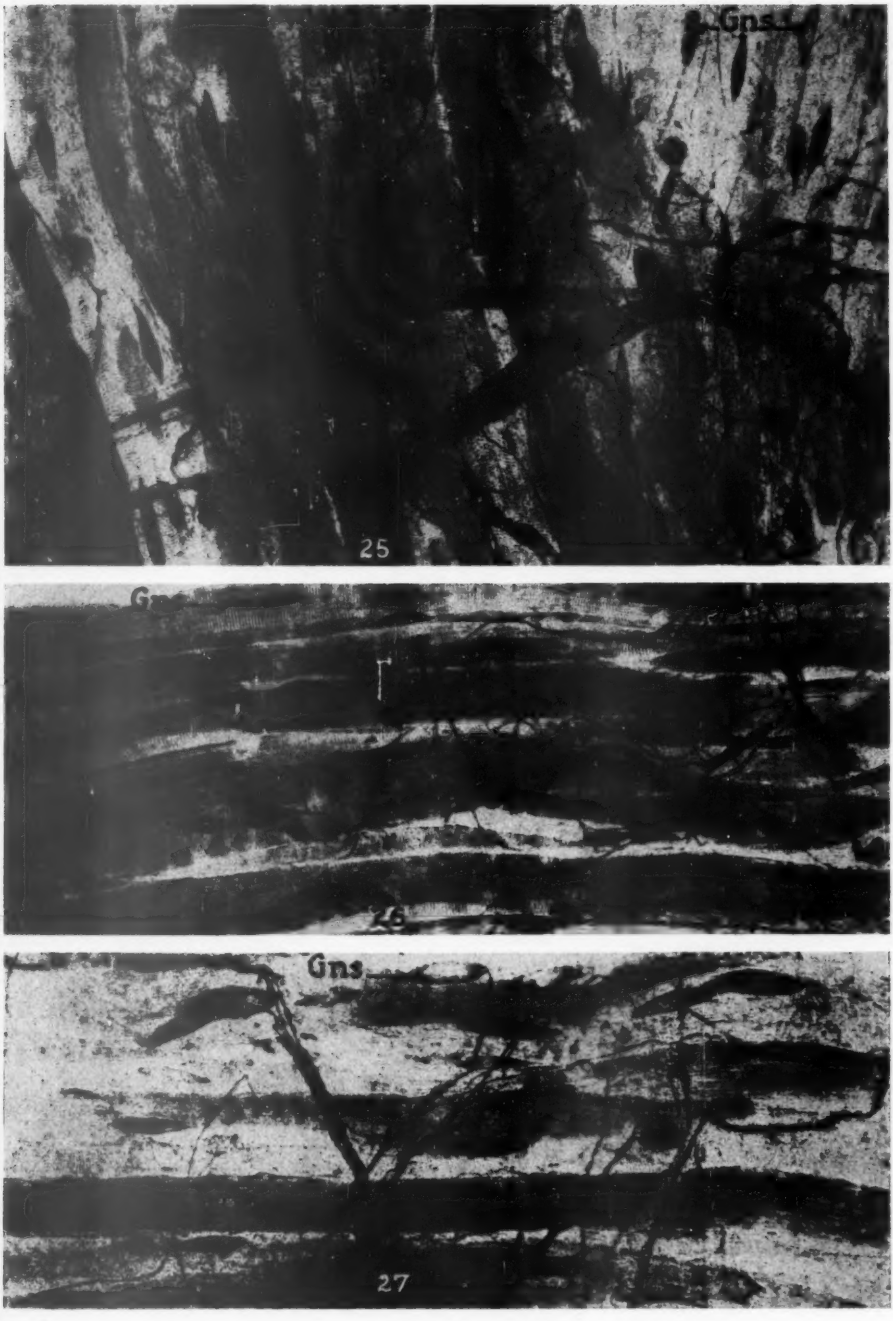
## PLATE 32

FIGS. 23 and 24. Innervation of gastrocnemius muscle of the rat 24 hours (Fig. 23) and 96 hours (Fig. 24) after tenotomy. There is a copious discharge of giant fusiform neurosomes (Gns) 96 hours after tenotomy (Fig. 24), as well as increased diameter and staining capacity of the epilemmal axons. The normal epilemmal axons (Fig. 8), and those at 24 hours (Fig. 23) after tenotomy, vary from 1 to 7 $\mu$ in diameter, whereas at 96 hours following tenotomy the axons vary in diameter from 1 to 20 $\mu$. Disuse of the innervation following tenotomy produces an enlargement of the axons of the nerve supply because of the delay in, or block to, the neurogenic discharge by disuse, through loss of normal stretch of the muscle fiber. Gold technic, teased whole muscle fibers. $\times$ 200.

## PLATE 33

FIGS. 25, 26, and 27. Innervation of the gastrocnemius muscle of the rat 15 days following tenotomy. The discharge, diffusion, and disappearance by hydrolysis of the giant fusiform neurosomes are greatly slowed by disuse and loss of normal stretch of the muscle fiber. Some of the pathologic neurosomes are hyperchrysophilic whereas others undergoing dissolution by hydrolysis are either hypochrysophilous or achrysophilous. The abnormal structural expression of the neurogenic transmitter substance is the result of retardation in the rate of the chemical influence of nerve on muscle. The structure of the pathologic chemical transmitter is more easily demonstrated than normally by slowing the rate of nerve impulses by disuse following tenotomy. The abnormal transmitter substance accumulates in quantity under slow motion and assumes, by streamlining, the shape of a torpedo that slowly diffuses and dissolves in the muscle substance. The drag or frictional resistance of the myoplasm is reflected in the turbulent eddies and distortions of the cross striations produced at the prow and in the wake of the travelling neurosomes (for comparison with Figs. 63 to 75). Gold technic, teased whole muscle fibers. × 200.



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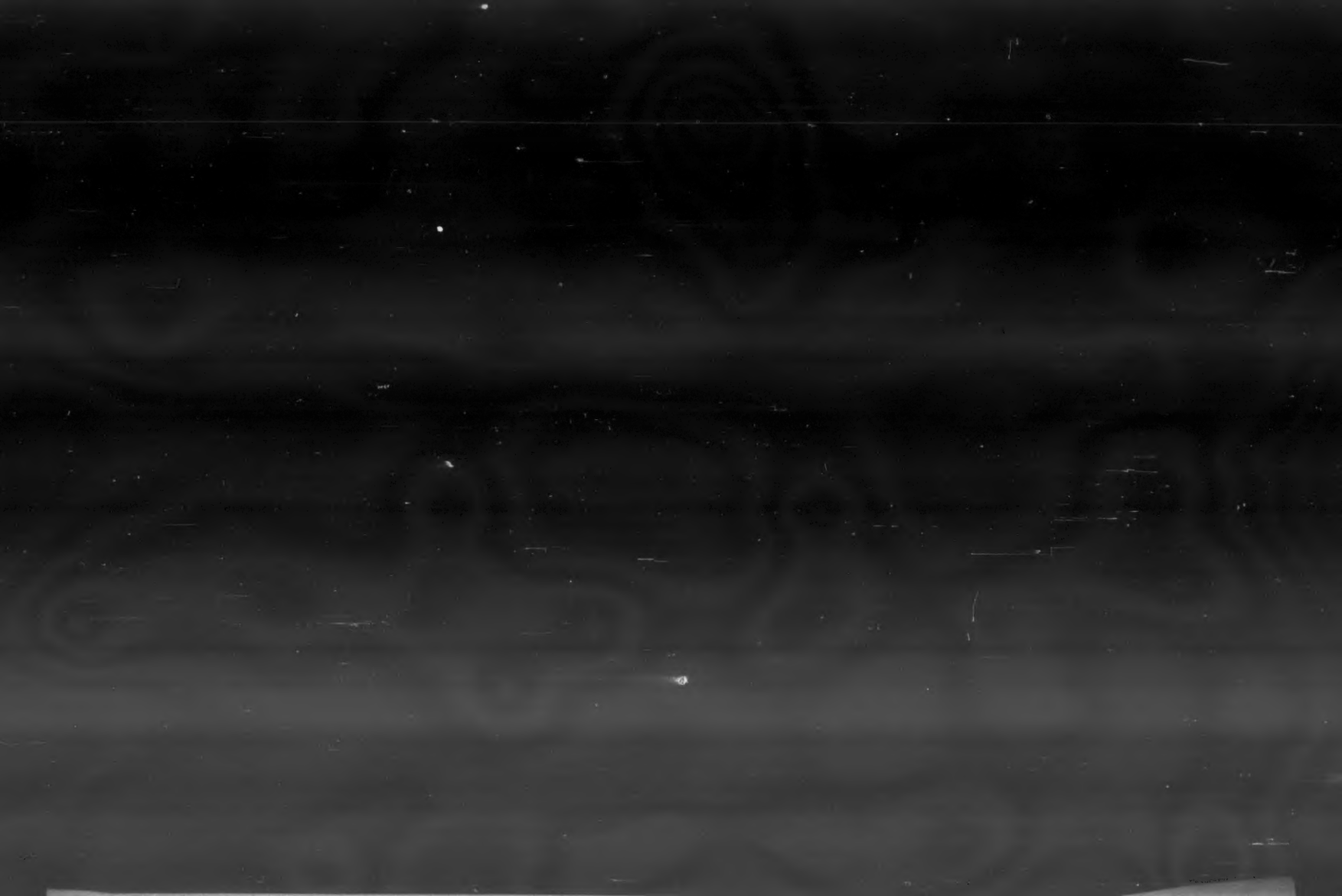
## PLATE 34

FIGS. 28 to 39. Normal (Figs. 30 to 32, and 35 to 38) and abnormal (Figs. 28, 29, 33, 34, and 39) variations in the structure of the motor nerve endings and epilemmal axons in the gastrocnemius muscle of the rat. The pleomorphism of the nerve ending appears to be produced by the neuroprotoplasmic streaming of ameboid motion of the terminal hypolemmal axons. The periodic discharge of normal granules of neurosomes produces the granules of the sole plate of Kühne (Kg). Some of these granules are produced also by the nuclei related to the normal nerve endings. These granules are usually increased in amount around the retracted endings and depleted around the expanded (Figs. 34 to 37) and exhausted (Fig. 38) endings. The engorgement and depletion of the epilemmal axons appear to result from the centrifugal conduction and peripheral discharge of neurogenic substances by microperistaltic waves. During the first week after tenotomy, the hypolemmal and epilemmal axons appear to be engorged with an increased amount of gold-impregnated material (Figs. 28, 29, 33, and 34). From the 7th to the 14th days, the motor end-plate usually takes a uniform impregnation with gold because of the increased amount of neurogenic material which it contains. In direct continuity with this pathologic nerve ending (Fig. 39, NS), there are projections of pathologic neurosomes which likewise are deeply impregnated with gold. Gold technic, teased whole muscle fiber. × 850.



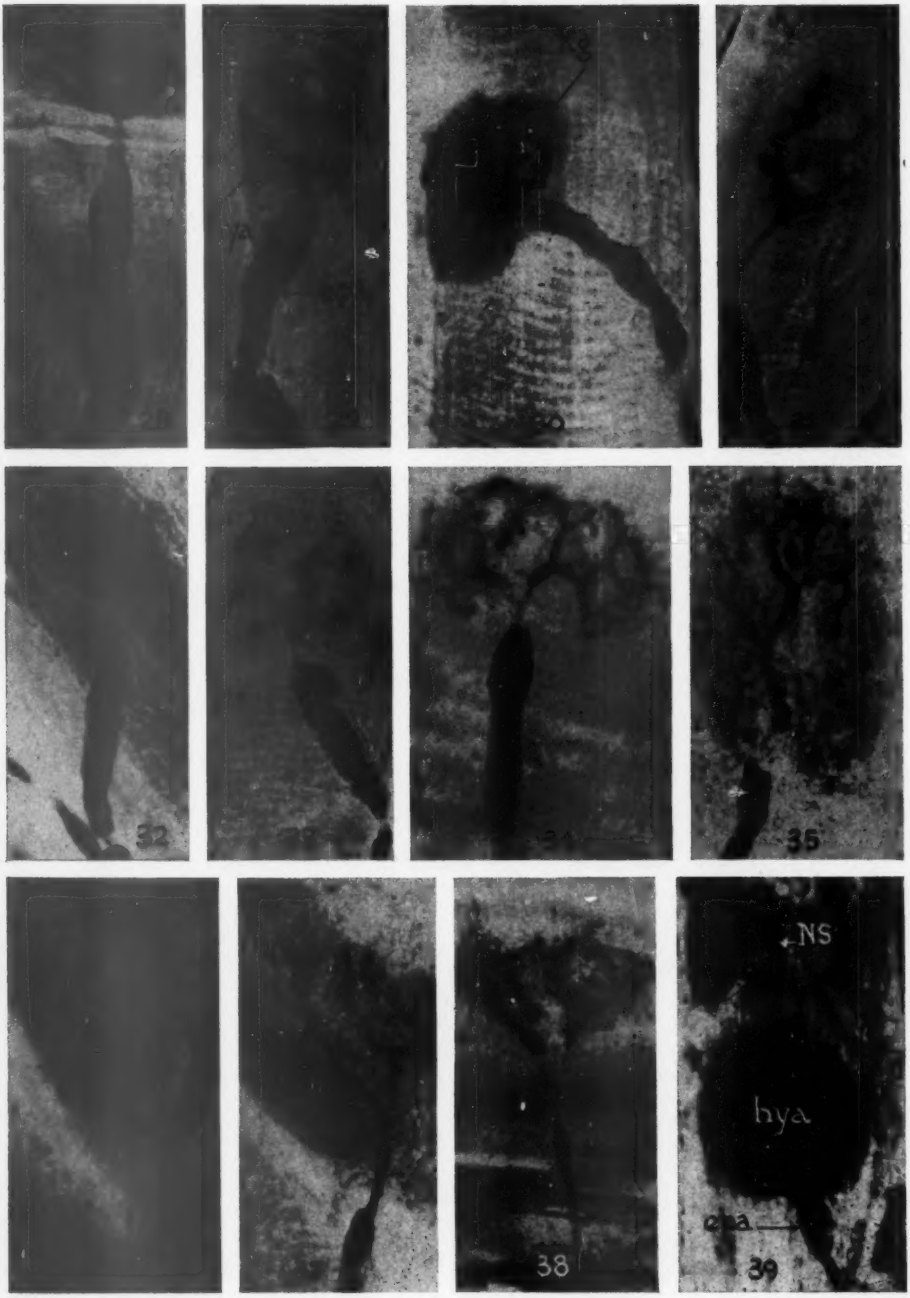

32
35
NS
hya
38
39

PLATE 35

FIGS. 40 to 46. Hyperchrysophilia of motor nerve endings in the gastrocnemius muscle of the rat 10 days after tenotomy. The densely impregnated nerve endings are spherical, oval, or fusiform. In some nerve endings there is a beginning discharge of a light cloud of granules (Figs. 43, 44, and 46) to the right of the nerve ending. The genesis of a giant neurosome is observed in the last nerve ending (Fig. 46, Gns). With silver impregnation after formalin fixation there is evident an increased number of nuclei around the motor end-plates as well as an increase in the number of the neurofibrils in the terminal axons. The significance of these changes manifested by silver is not evident without a comparison with the morphologic changes revealed by the teasing of whole muscle fibers after gold impregnation. The latter method is better than the former to demonstrate that the motor end-plate is a microscopic gland of internal secretion, the structure of which varies with the phases of secretion. Gold technic, teased whole muscle fibers. × 850.

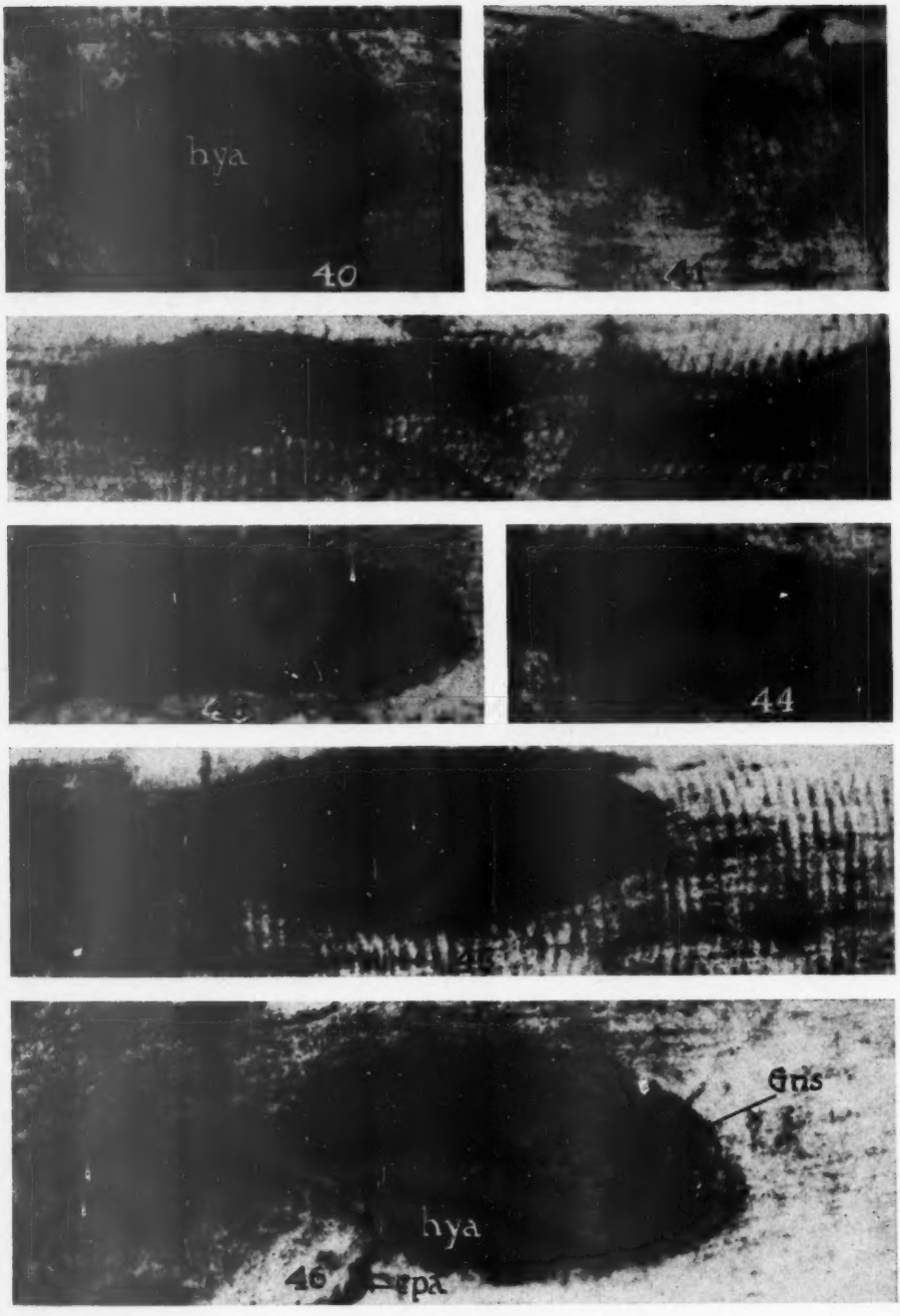



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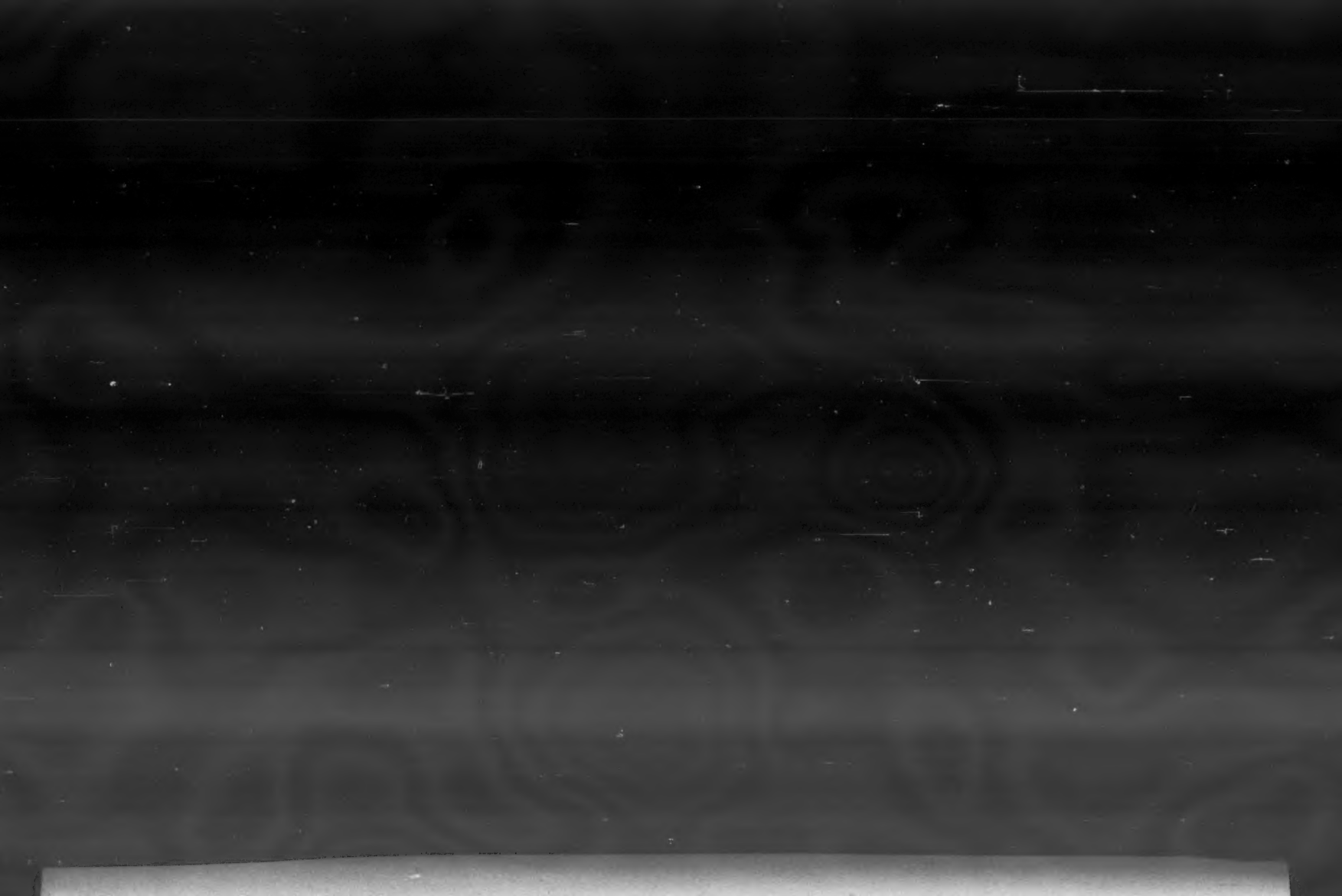
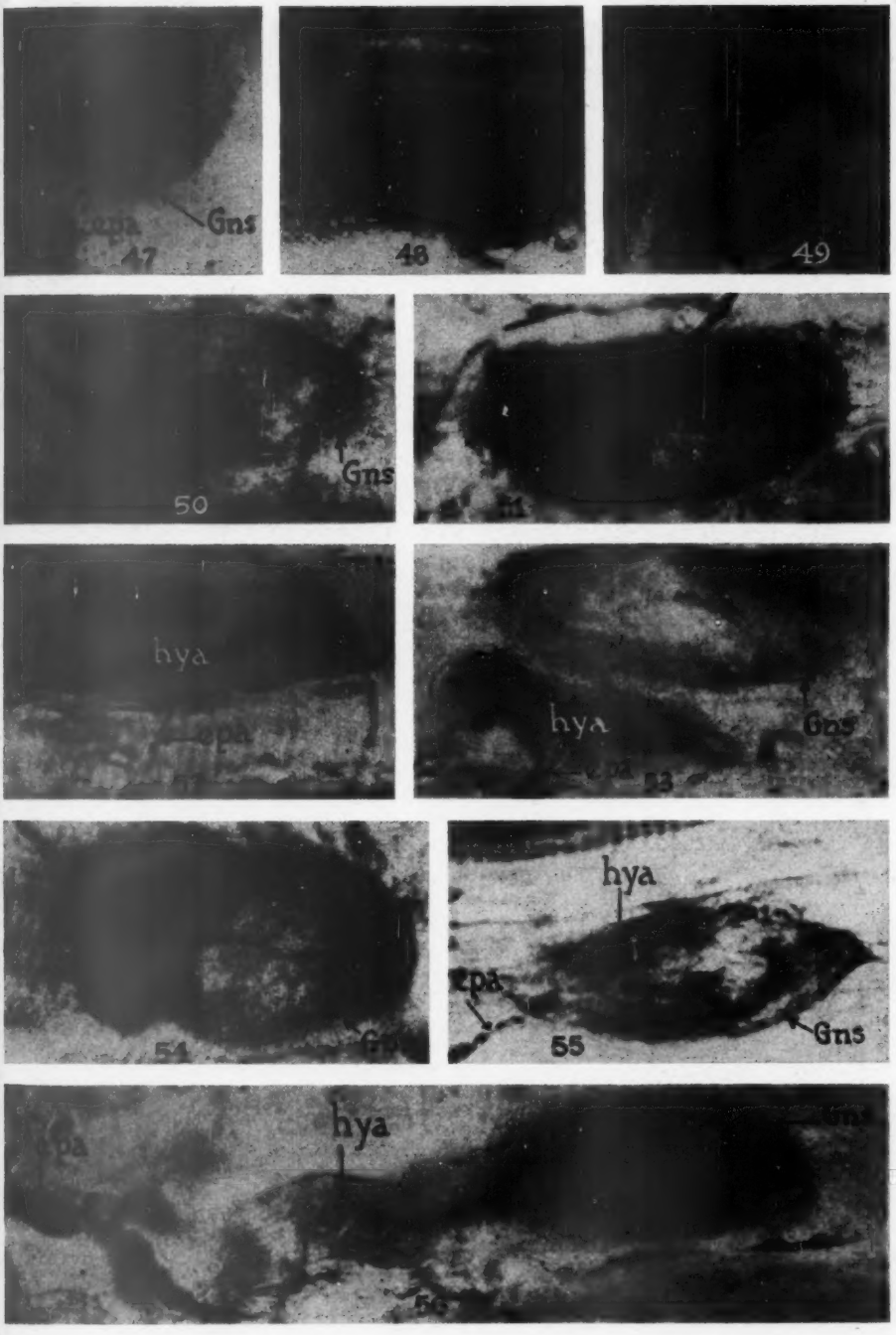
PLATE 36

FIGS. 47 to 56. The genesis of giant, globular and hemispherical pathologic neurosomes (Gns) from nerve endings in the gastrocnemius muscle of the rat 12 days after enclosing the right hind leg in a plaster of Paris cast. The mushroom eruption of the light cloud of granules from two nerve endings (Figs. 52 and 53) has a microscopic structure roughly comparable to the cloud of eruption produced by the explosion of the atomic bomb at Bikini in 1946. There is a complete transformation of some terminal nerve endings into a fusiform mass of granules (Figs. 55 and 56). The morphologic changes in muscle by immobilization of the limb in a cast are quite comparable to those produced by tenotomy. The neurosomic masses discharged during the early stages of disuse produced by a cast are usually more globular and oval than those produced subsequent to tenotomy. This mechanical deformation of the giant neurosomes may be dependent upon the differential rates of atrophy of muscle following the two methods of inducing disuse. Gold technic, teased whole muscle fibers. × 850.



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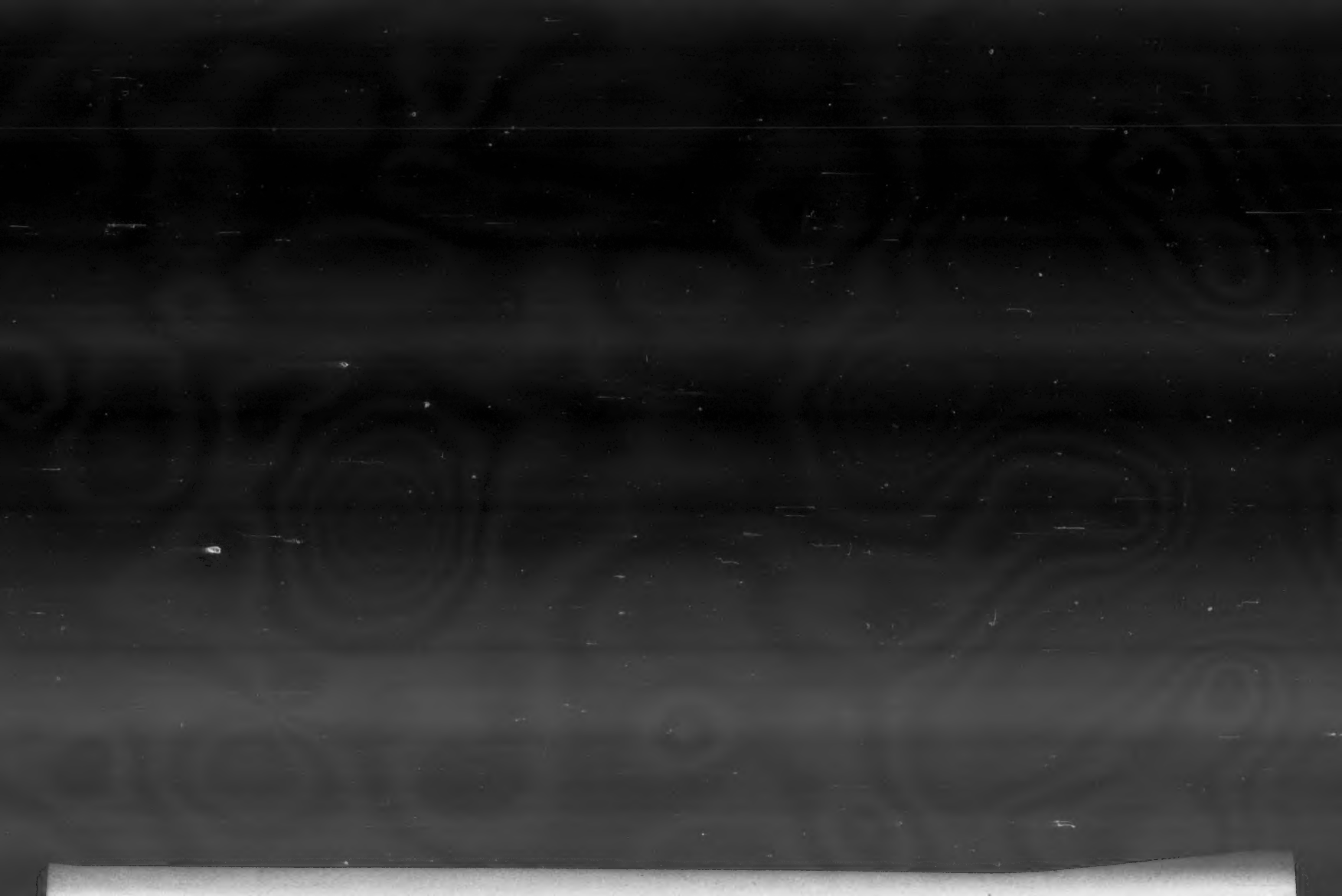
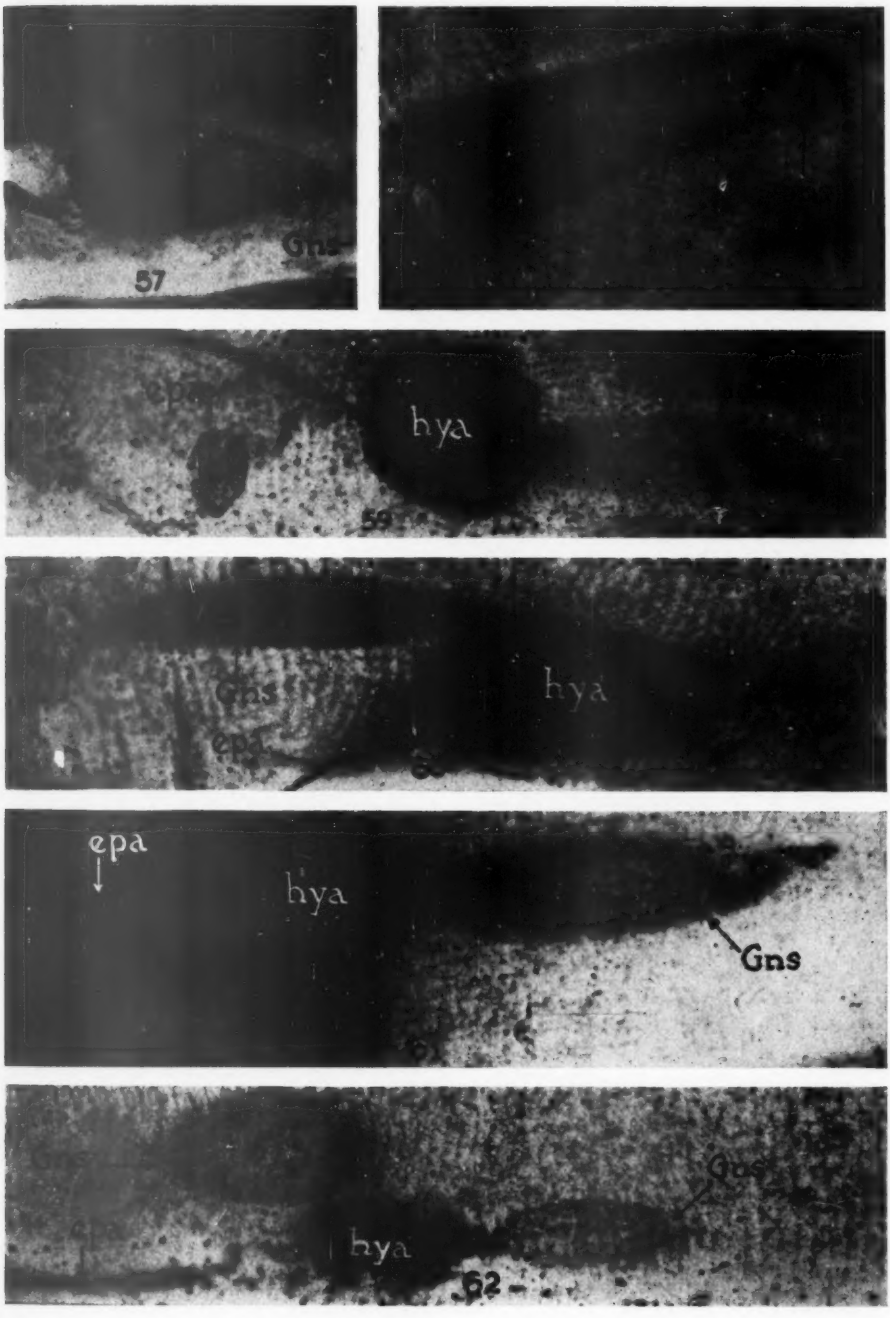
## PLATE 37

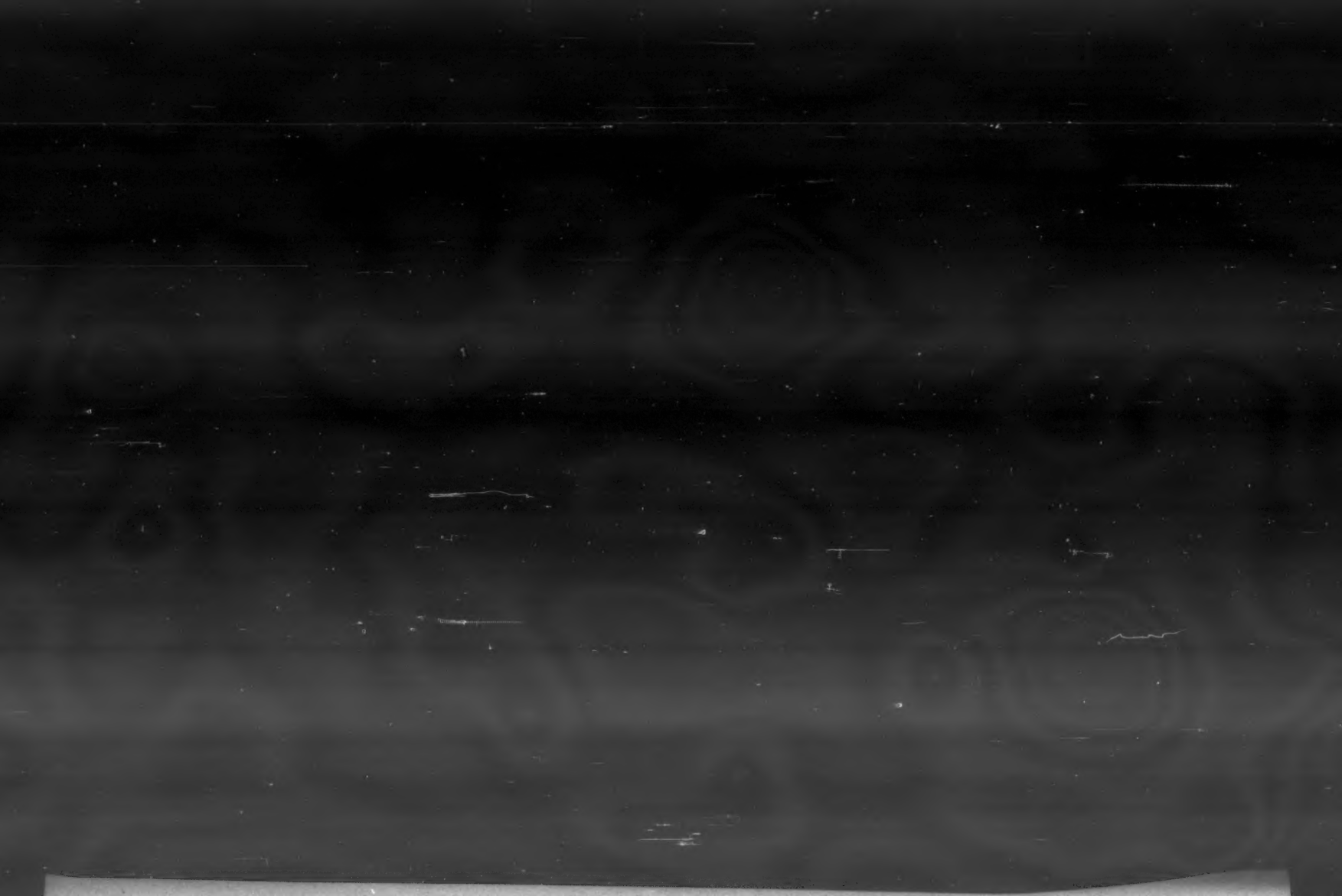
FIGS. 57 to 62. The genesis of elongated, giant fusiform neurosomes (Gns) from motor nerve endings in the gastrocnemius muscle of the rat 15 days (Figs. 57 to 61) and 1 day (Fig. 62) after tenotomy. There are progressive stages in the accumulation, projection, and discharge of these neurosomes retarded in rate of secretion by the disuse of the muscle following tenotomy. Some of these neurosomes are intensely impregnated with gold, whereas others take the gold impregnation very lightly. The slowing in the rate of discharge of nerve impulses into muscle by disuse determines the increase in the quantity of the neurogenic secretion observed at and around the nerve terminal. The visualization by photography of the pathologic nerve impulse is, therefore, determined by the accumulation of neurogenic substances produced by the retardation in the rate of discharge, diffusion, and dissolution by hydrolysis of the neurogenic secretion from the motor nerve endings. Gold technic, teased whole muscle fibers. × 850.




Gns
57
hya
59
Gns
hya
epa
epa
hya
Gns
Gns
hya
62

## PLATE 38

FIGS. 63 to 69. Giant fusiform neurosomes (Gns) in the myoplasm of the gastrocnemius muscle fibers of the rat 15 days after tenotomy. Friction of the myogenic fluid slows down the transmission of the giant neurosomes. The resisting drag of the myoplasm on the moving neurosomes is associated with the formation of whirlpools or eddies. These turbulent eddies trail behind the neurosomes. The streamlining of the neurosomes results in tapering ends. The pointed rear end is more important in streamlining than the front end and in some locations (Fig. 65) neurosomes assume the "tear-drop design" of streamlining with one end blunt and convex like that of a torpedo. This design is modified, however, by the fact that the neurosome is not an inert particle moving in the muscle, but is subjected to colloidal interaction and undergoes progressive incorporation in the muscle. The resulting eddies or vortices are detected by the distortion of the related cross striations at the prow and in the wake of the giant neurosomes. These neurosomes undergo progressive dissolution (Figs. 68 and 69) by hydrolysis, and their granules become incorporated into, and aligned with, the cross striations of the muscle. Gold technic, teased whole muscle fibers. × 850.



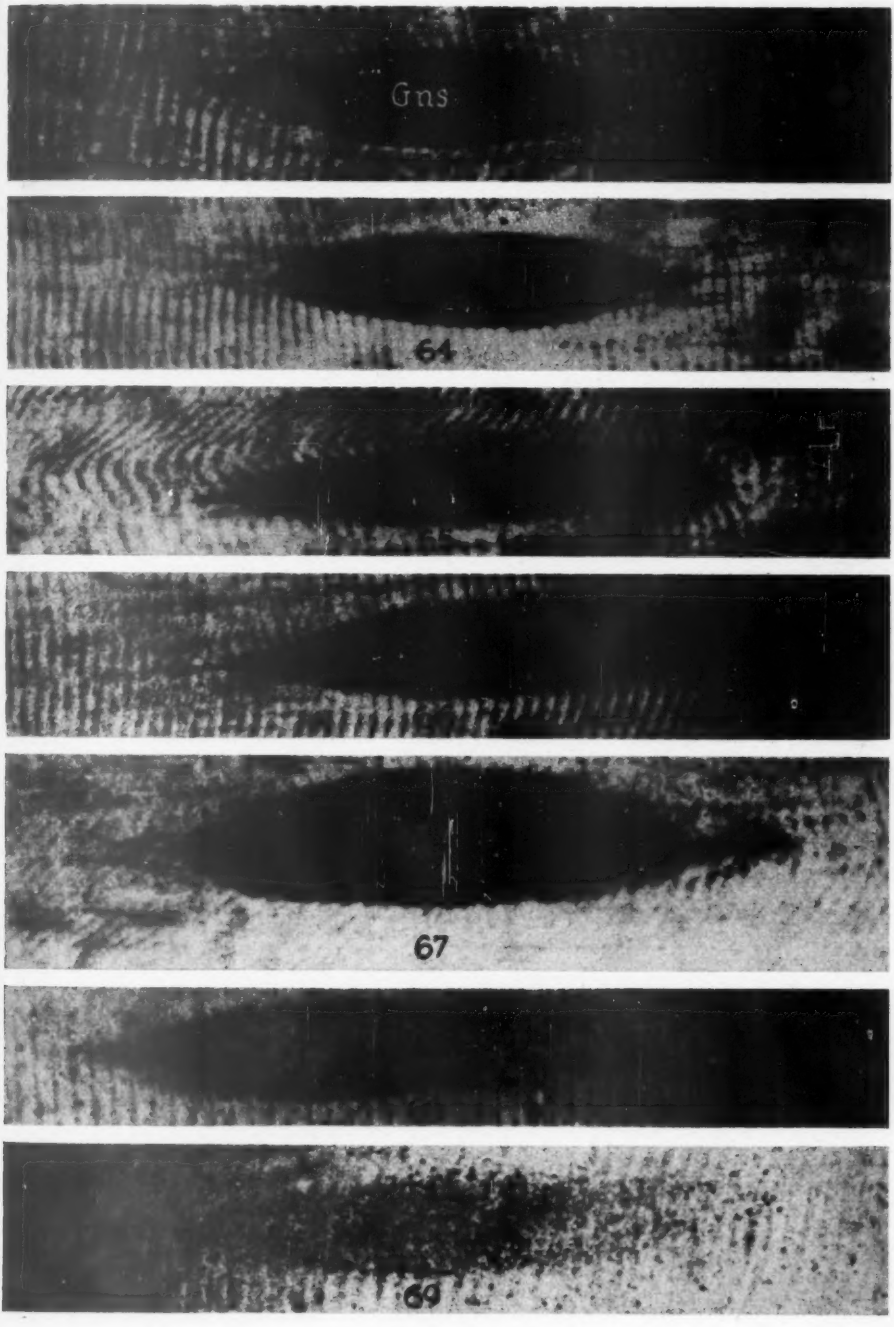



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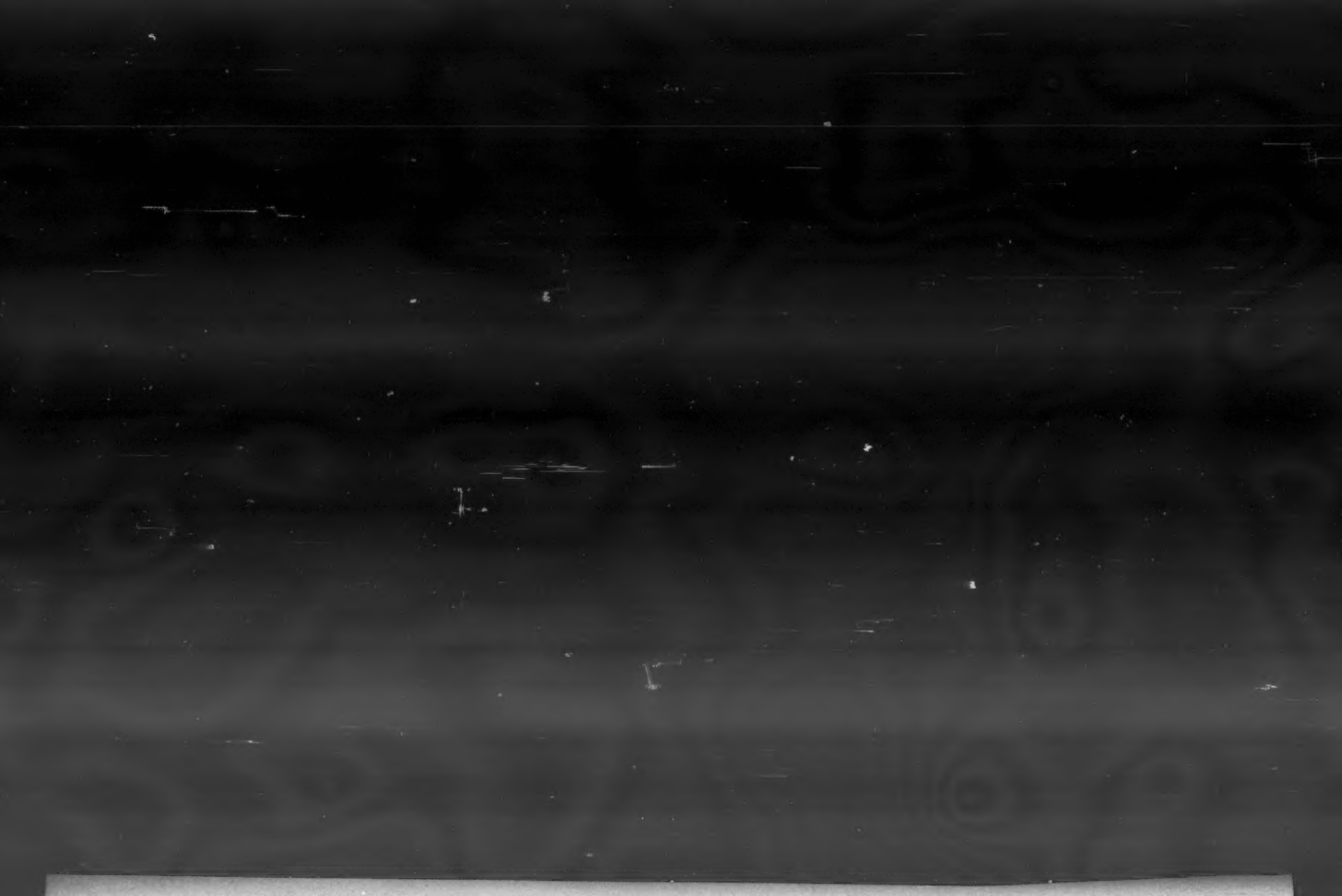
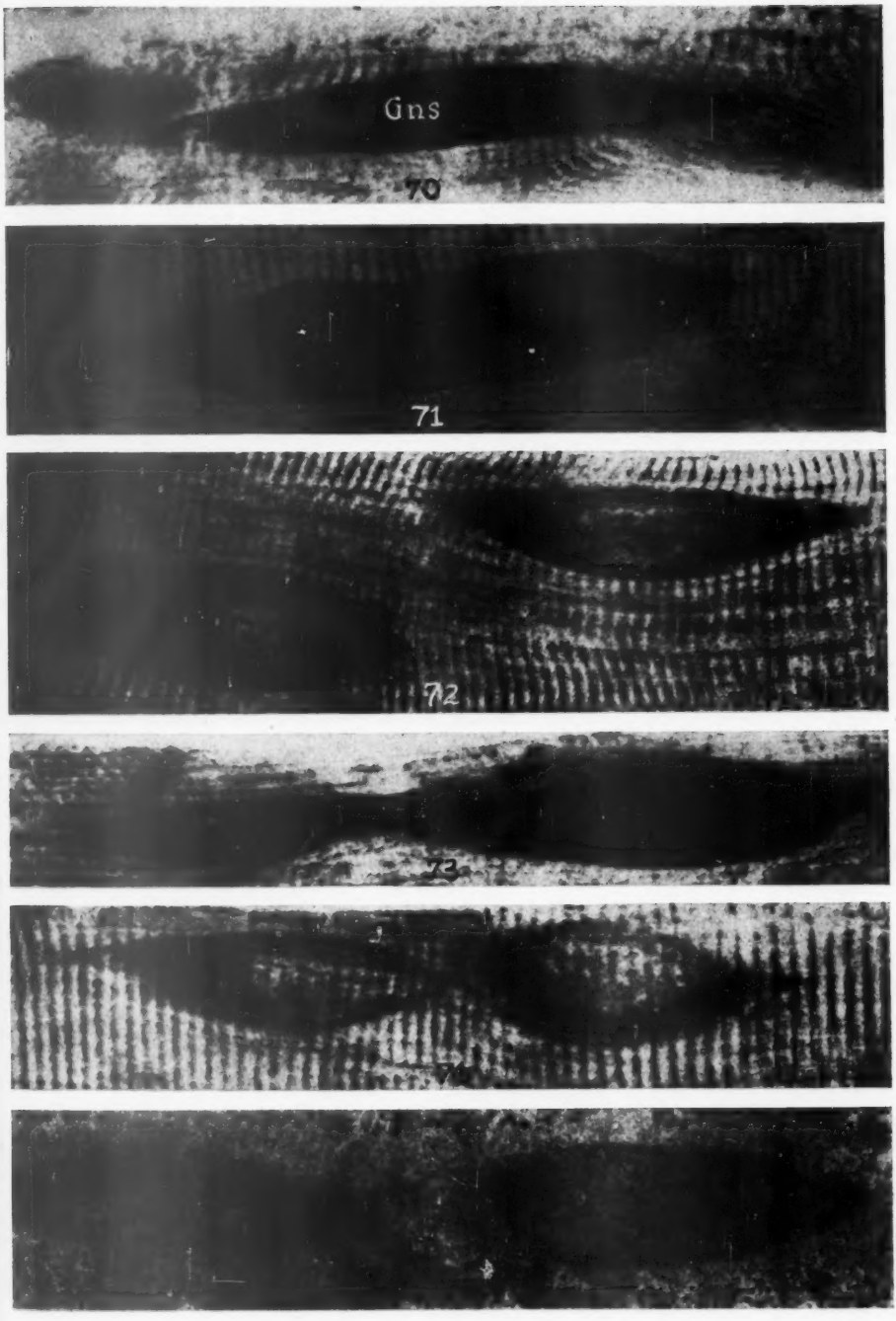
PLATE 39

FIGS. 70 to 75. Single (Fig. 70) and duplex giant fusiform neurosomes (Gns, Figs. 71 to 75) in the gastrocnemius muscle of the rat 18 days after tenotomy. Some neurosomes are hyperchrysophilous (Figs. 70 and 71). Others, in different degrees of dissolution, are hypochrysophilous (Figs. 72 to 75). These neurosomes gradually disappear by hydrolytic granulation, and their granules become aligned with the striations of the muscle. The structural expression of these pathologic neurosomes in muscle is the resultant of a slowing down in the rate of diffusion and dissolution of neurosomes accompanying the retardation in rate and consequent increase in mass of the neurosomes that parallel the pathologically slow rate of nerve impulses in muscle undergoing atrophy of disuse. The real function and significance of the structure of the normal neuromuscular apparatus are revealed by retarding the rate of the normal discharge, diffusion, and dissolution of the neurosomes that accompany the normal electric signs of the nerve impulses. Gold technic, teased whole muscle fibers. × 850.




Gns
70
71
72
73

# VASCULAR PROLIFERATIONS, WITH FEATURES OF ARTERIOVENOUS ANASTOMOSES, IN THE SYMPATHETIC CHAIN OF HYPERTENSIVE PATIENTS *

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In the course of the routine histologic study of large numbers of thoracolumbar sympathetic ganglia removed surgically in patients with essential hypertension, peculiar vascular structures of organoid appearance were observed repeatedly. It is the object of this paper to describe these findings, and to discuss their possible significance.

## MATERIAL

The observations reported here have been made on specimens from thoracolumbar sympathectomies performed on hypertensive patients. The portions removed usually included the area from the second or third thoracic ganglion to the second or third lumbar ganglion. However, because the specimens were not always removed in one piece, a proper identification of the provenance of an individual ganglion was often impossible. As control material, the sympathetic chains of 5 patients who had died from diseases other than essential hypertension were obtained at autopsy. The respective ages of these patients were 2 days, and 11½, 26, 35, and 52 years. For the majority of the cases, the ganglia were fixed in Zenker's solution, and the paraffin sections were stained with hematoxylin and eosin, Masson's trichrome stain, resorcin stain for elastic fibers, and Wilder's stain for reticulum. A few of the specimens, after fixation in chloral hydrate, were stained with the Cajal silver nitrate method. Some were fixed in Orth's fluid and stained with the method of Schmorl. In 8 cases, all of the ganglia removed by the surgeon were studied in serial sections. In general, however, the paraffin blocks were cut at three or four different levels, but no complete serial study was made.

## MICROSCOPIC FINDINGS

In a fraction of the cases examined, the sympathetic ganglia or the immediately adjacent nerve trunks showed the presence of well defined, usually round bodies, up to 250 $\mu$ in approximate diameter, situated either in the substance or near the capsule of the ganglion,

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and consisting of a complex system of narrow, tortuous blood channels arranged in glomerular * fashion, associated with an arteriole and connected with efferent venous sinusoids (Figs. 1 to 7). The small vessels of these vascular bodies were lined by large endothelial cells that could be remarkably swollen so that the respective lumina often were inconspicuous. The more or less abundant intervening stroma contained varying numbers of elongated or round elements with oval, vesicular nuclei larger than those of the adjacent endothelial cells. The cytoplasm of these extra-endothelial cells as a rule was clear; it might contain small eosinophilic granules but no definite myofibrils. These extravascular elements were at times (but not always) wrapped around part of the circumference of the small vessels of the bodies. In other instances they appeared to be scattered in the stroma between the vascular loops.

The arteriole associated with these structures showed, in general, a marked hypertrophy of its muscular coat, and often hyalinization and degeneration, even to a marked degree. In most of the cases, however, the small vessels of these structures seemed to sprout directly from the wall of the associated arteriole and to communicate with its lumen (Figs. 6 and 7). Schmorl stains for chromaffin substance have been negative in these bodies, and no relationship appears to exist between them and the not infrequent finding of masses of chromaffin tissue in the vicinity of the ganglia. The study of a possible innervation of these small organoid structures has been difficult because to date I have found no way of predicting, through clinical data or gross inspection, which of the ganglia will show positive findings. However, after sectioning serially several ganglia prepared with the Cajal method, some vascular glomeruli were identified. The photomicrograph (Fig. 5) shows that the silver impregnation has been successful but that the body itself contains no axons. Regressive changes have been observed in some of the glomerular structures, and they consist in general of foci of fibrinoid degeneration and necrosis similar to those observed in the walls of the arterioles in malignant hypertension. These are interpreted as diseased arteriolar segments.

As has already been mentioned, the microscopic findings in some of the cases suggest that the tortuous capillaries and the intervening extra-endothelial cells (pericytes?) composing these glomerular vascular structures take their origin from the adventitia of diseased arterioles. These appear to be surrounded by a highly cellular zone, in

* The term "glomerulus" or "glomerular structure" used here and in the following paragraphs is not intended to imply any resemblance to the renal glomerulus, nor to the neuromyo-arterial glomus. It is used in its original literal meaning of "small body."

which there are narrow capillaries and large irregular cells with vesicular nuclei, similar to those seen, and already described, in the fully developed vascular bodies (Figs. 8 and 9).

Furthermore, in some recent favorable cases in which the vascular glomerular structures could be studied three-dimensionally by means of serial sections, it appeared that some of the associated arterioles were segmentally involved by severe necrotizing arteriolar sclerotic disease, and that the proliferation of small blood vessels and accompanying pericytic cells was originating from the segments of the arteriole immediately adjacent to the involved portion.

Before ending this description, mention should be made of the venous circulation in the sympathetic ganglia. This appears to consist exclusively of sinusoidal vessels, with prominent endothelial lining, and no muscular coats. These sinusoids are often seen to be situated alongside of, and in direct contact with, the muscular coats of the adjacent small arteries and arterioles. It is in these venous sinusoids that the blood from the capillary system of the ganglion is collected (Fig. 10). The existence of similar large venous sinusoids in the sympathetic ganglia of rabbits was recognized and described by Ranvier in 1875. The probable importance of this type of sinusoidal circulation in the mechanism of formation of the vascular glomerular structures will be discussed below.

## Incidence

Observations were made from specimens from 95 thoracolumbar sympathectomies. Vascular glomerular structures were found in 9 patients, in 3 of which the bodies were found in the ganglia of each side. Ganglia containing multiple bodies were found four times: two contained two bodies, one four, and another five. These findings make no pretense of statistical accuracy, as serial sectioning was used only in a few of the specimens.

## Relationship Between Presence of Vascular Bodies and Clinical Features

The 9 patients in whom the vascular glomerular structures were found were all in advanced stages of arteriolar sclerotic hypertension. The average age was 35.11 years. There were 7 females and 2 males. The average duration of the disease was 7.5 years. All of the patients complained of subjective symptoms consisting of headaches, nervousness, dizziness, and often blurred vision. In 7 the eyegrounds showed sclerosis of the arterioles, arteriovenous nicking, and old and recent hemorrhages. In 2, no record of ophthalmologic examination was found in the clinical chart. At least traces of albumin were found in the urine

of all. The urea clearance, and the pitressin concentration tests showed definitely impaired renal function in 4, and apparently in only one case was the function of the kidney within normal limits.

## Discussion

The objects under consideration are well defined, complex vascular bodies of organoid structure which appear to be interposed between vessels of arterial and of venous type. The associated arteriole is in general affected by advanced arteriolar sclerosis. Between the small tortuous capillaries of the "glomeruli" there are varying numbers of large, clear, extra-endothelial elements resembling the pericytes studied originally by Zimmermann and more recently by Murray and Stout (Figs. 2, 8, and 9). Some of the histologic findings seem to indicate that the vascular elements in question originate from a proliferation of small blood vessels and of pericytic cells which, starting in the adventitia of diseased arterioles, connects them with large venous sinusoids.

To the best of my knowledge, no similar structures have hitherto been described, either for normal or pathologic human sympathetic ganglia. Furthermore, the personal study of serial sections of the stellate ganglion and of the thoracolumbar sympathetic ganglia of 5 patients dying from causes other than essential hypertension failed to reveal any comparable finding. Only one paper could be found in the available literature which appeared to offer a possible lead in the interpretation of these findings. This is an article by Nonidez, in which he described in great detail his observations of simple and multiple arteriovenous anastomoses in the stellate and thoracic ganglia of young dogs, believed by him to belong in the same class with the glomus coccygeum and the peripheral neuromyo-arterial glomus of Masson. These are organoid structures consisting of multiple arteriovenous channels surrounded by conspicuous clear epithelioid cells which are richly innervated by efferent and afferent fibers. Nonidez suggested that they may represent organs for the regulation of the blood pressure. No similar structure appears to exist in the normal human sympathetic ganglia.

The structures described in this paper and the bodies of Nonidez in the dog appear to have only a superficial resemblance. Both have an organoid architecture and consist of a complex network of blood vessels interposed between an arteriole and efferent venous sinusoids. However, in the structures described by Nonidez, the vascular channels of the multiple anastomoses show orderly arranged, perivascular, clear epithelioid cells interpreted by him as modified smooth muscle cells. In the structures illustrated in this paper, extra-endothelial cells of peri-

cytic type are present, but are not as conspicuous nor arranged in as orderly a fashion. Furthermore, the rich innervation described by Nonidez in the structures studied by him could not be demonstrated in my cases. This seems to me to be a strong point against considering the present findings as structures of the neuromyo-arterial glomus type.

In my opinion, the occurrence of these vascular bodies is closely related to the presence of severe arteriolar sclerotic disease. In fact, this is strongly supported by some of the findings illustrated in the preceding paragraphs, and by the absence of similar structures in the ganglia of nonhypertensive persons. The vascular channels of these glomerular formations appear to represent a short cut between the diseased arterioles and groups of large venous sinusoids. Because of these features, they can be rightly considered as arteriovenous anastomoses, occurring in areas where the normal blood flow has been interfered with by pathologic changes in the local arteriole. That new arteriovenous anastomoses can appear in regions subjected to various types of irritation with disturbance of the blood flow has been conclusively demonstrated by Clark. Under the conditions described in my material, the appearance of arteriovenous anastomoses may represent an attempt to shunt the blood into the adjacent large sinusoids which constitute the venous system of the ganglia, and from here to redistribute it to the ganglion itself, through the numerous capillaries which open into these sinusoidal vessels (Fig. 10). Therefore, the observations of Nonidez on the dog acquire interest in the discussion of my findings. In fact, the presence of complex arteriovenous anastomoses in the sympathetic ganglia of other vertebrates may lead one to speculate on the existence of analogous but rudimentary structures in the human sympathetic ganglia from which the vascular bodies described above are derived. If morphologic peculiarities in the blood vessels of human sympathetic ganglia could be demonstrated, the presence of the vascular bodies in the ganglia and their absence in other organs or tissues of hypertensive persons could be understood. Search for such structures should be made either with injection technics, or with a three-dimensional model of the blood vessels of this region.

## Summary

Peculiar vascular proliferations, with features of arteriovenous anastomoses, have been observed in the thoracolumbar sympathetic ganglia of patients with severe essential hypertension. Their occurrence in sympathetic ganglia, and not in other organs or tissues of the hypertensive patient, is possibly related to some as yet unknown structural specialization of the blood vessels of this region.

I wish to express my gratitude to Dr. Maurice N. Richter and to Dr. Arthur Purdy Stout for their helpful suggestions and constructive criticism in the compilation of this paper. I wish also to express my thanks to Miss Evelyn Paget for her skillful histologic preparations.

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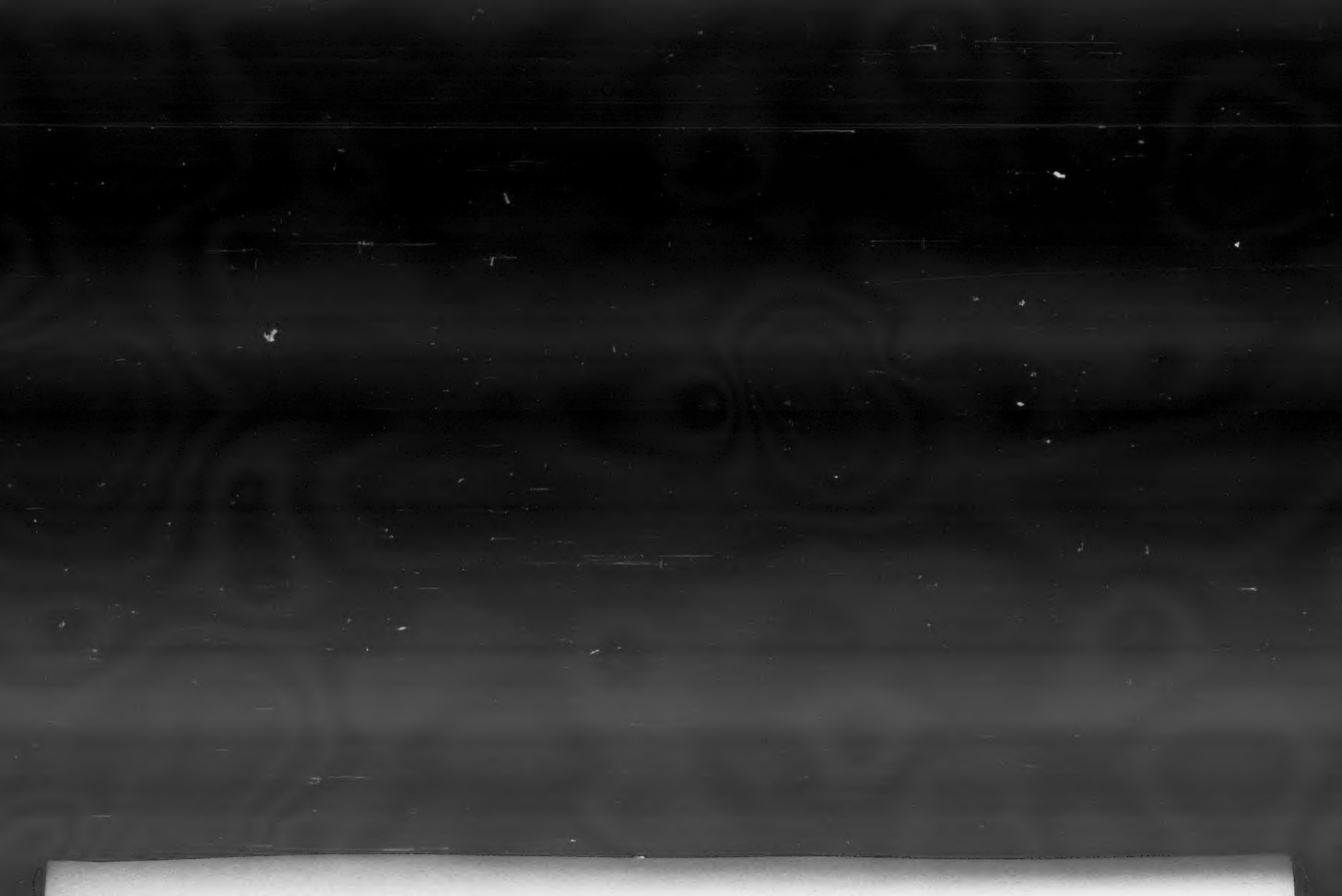
## DESCRIPTION OF PLATES

### PLATE 40

FIG. 1. Photomicrograph showing two vascular glomerular structures near the capsule of a sympathetic ganglion from a case of essential hypertension. Hematoxylin and eosin stain. × 115.

FIG. 2. Higher magnification of one of the "glomeruli" seen in Figure 1. On the left side may be seen an arteriole surrounded by clear, large, smooth muscle elements and three large venous sinusoids into which some of the narrow vessels of the vascular body open. Of note also are the numerous extra-endothelial cells between the vascular loops of the body. Hematoxylin and eosin stain. × 550.



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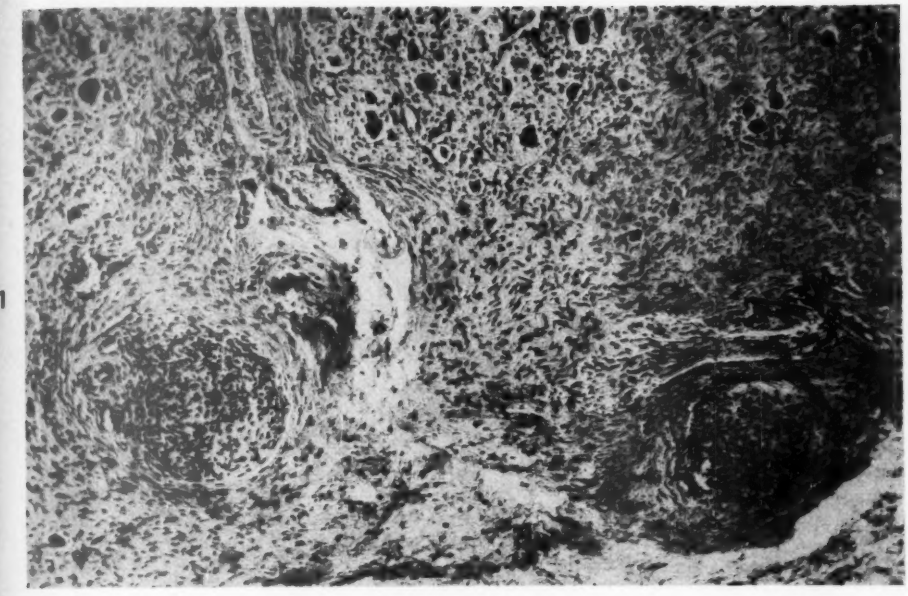

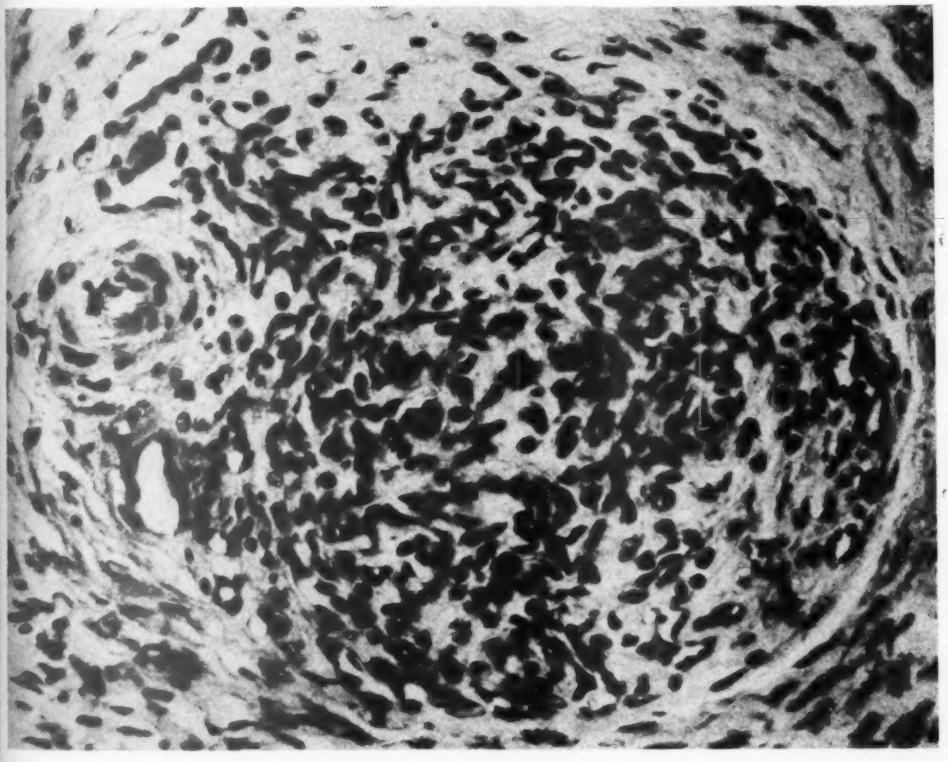

Lattes Arteriovenous Anastomoses in the Sympathetic Chain

PLATE 41

FIG. 3. Glomerular structure at the periphery of a sympathetic ganglion, showing a well defined expansion zone of the stroma. Several cross sections of a sclerotic arteriole, and a group of vessels, some of which are surrounded by pericytic elements, may be seen streaming toward a large thin-walled vessel in the upper portion of the photomicrograph, probably the collecting vein of the "body." Hematoxylin and eosin stain. × 300.

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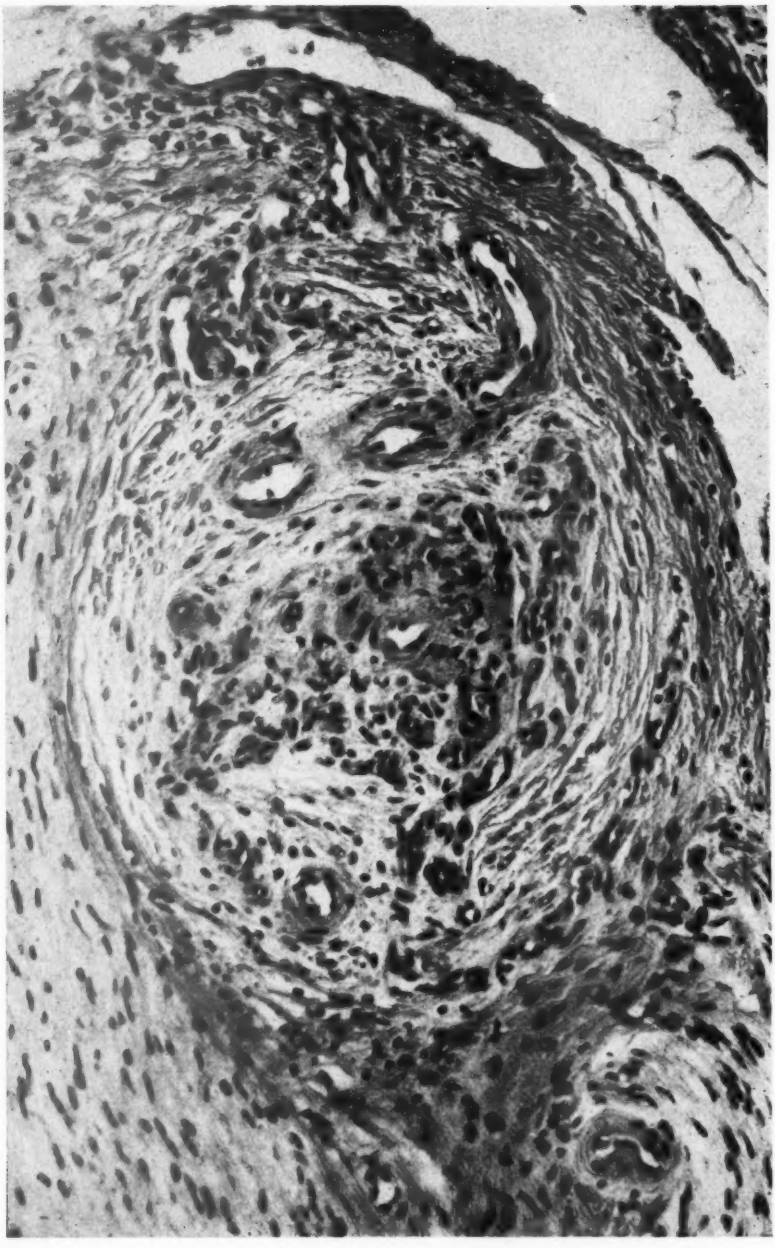

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Lattes Arteriovenous Anastomoses in the Sympathetic Chain

PLATE 42

FIG. 4. Photomicrograph of two succeeding sections, stained respectively with hematoxylin and eosin and Wilder's silver stain for reticulum, of a vascular body found in the center of a ganglion. Of note are the complete encapsulation of the body and the partial hyalinization of the associated arteriole. The silver stain demonstrates the regular pattern of the reticulum. × 255.

FIG. 5. Vascular glomerular structure in a sympathetic ganglion prepared by the method of Cajal. Axons are absent in the vascular body. × 500.

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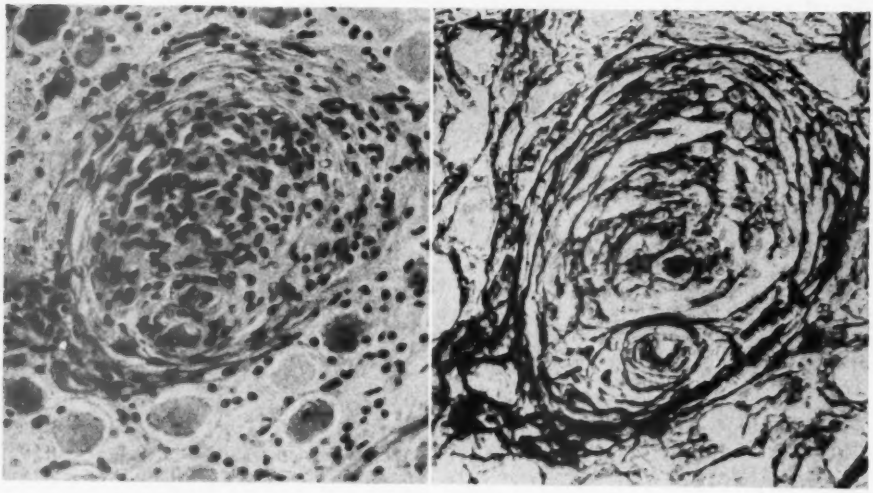

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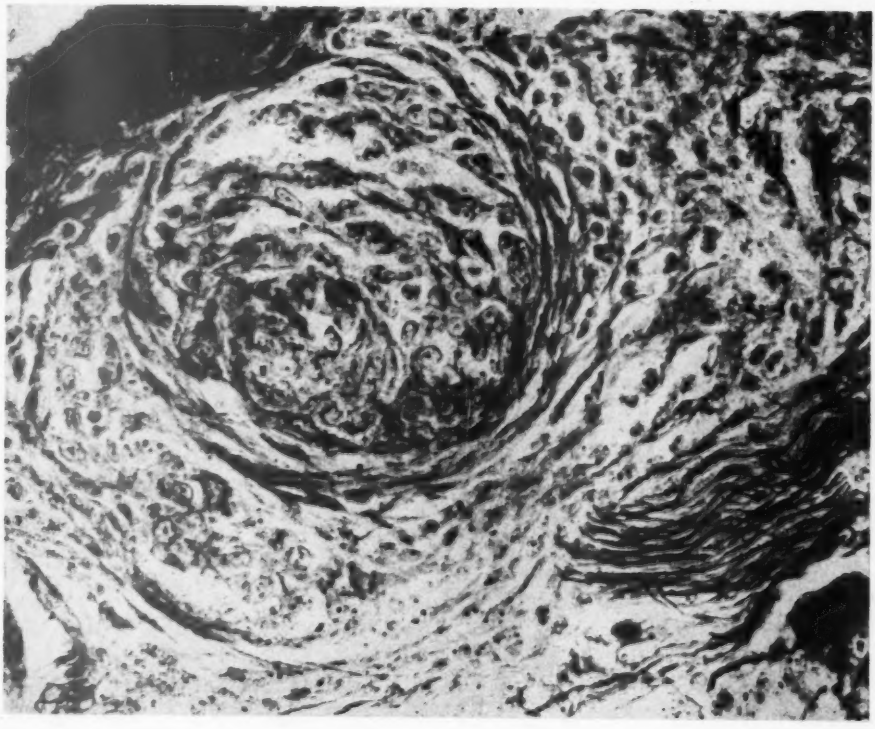

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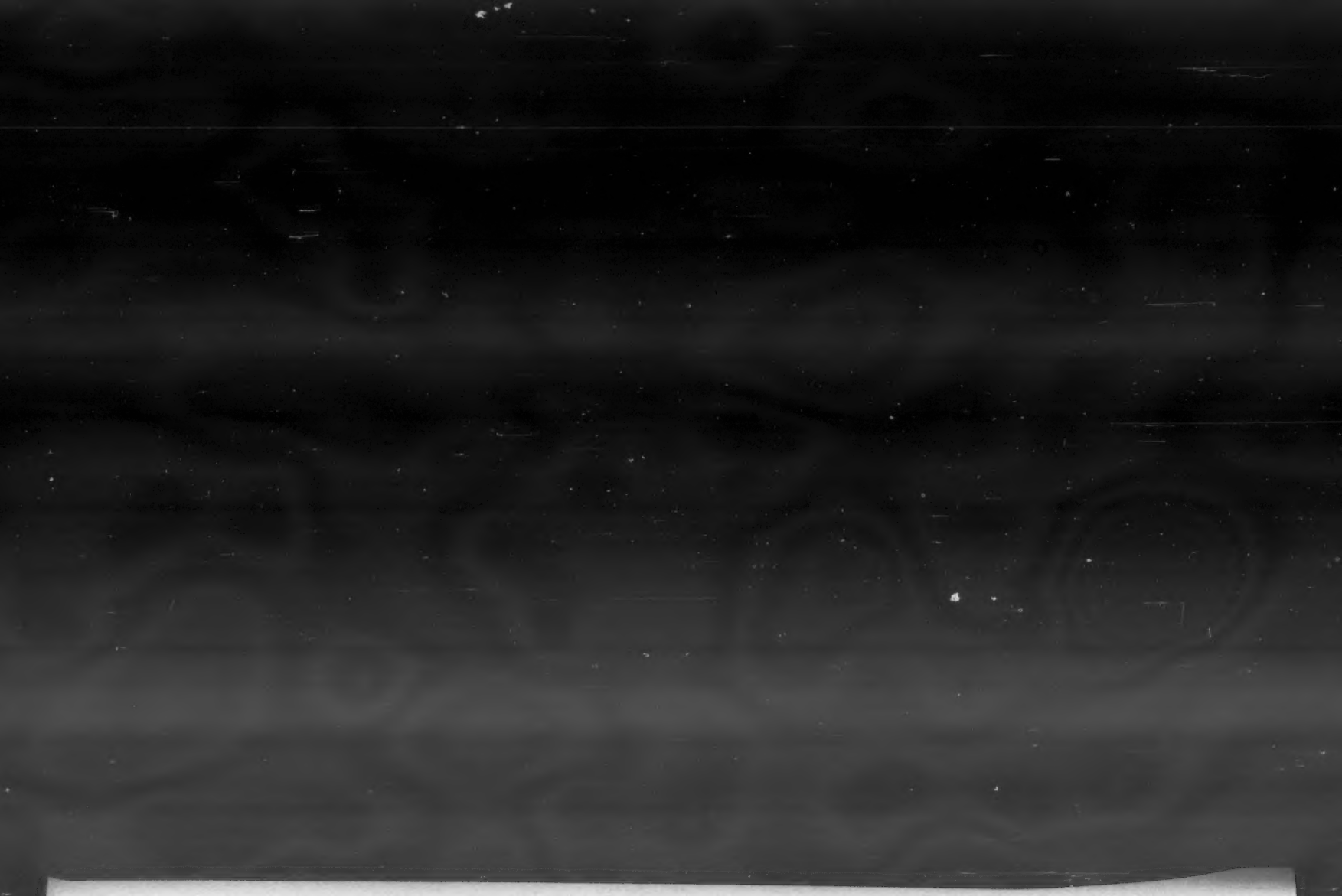
PLATE 43

FIG. 6. Composite picture showing every third section of a vascular body studied serially. There is a close relationship between the wall of the associated, tortuous, thick-walled arteriole and the vascular body. Section *e* clearly demonstrates a communication between the lumen of the arteriole and the vascular network of the body. (For additional details of this section see Fig. 7.) Of note also are the venous sinusoids at the periphery of the vascular body, into which the vessels of the body can be seen to open at different levels. Hematoxylin and eosin stain. × 140.



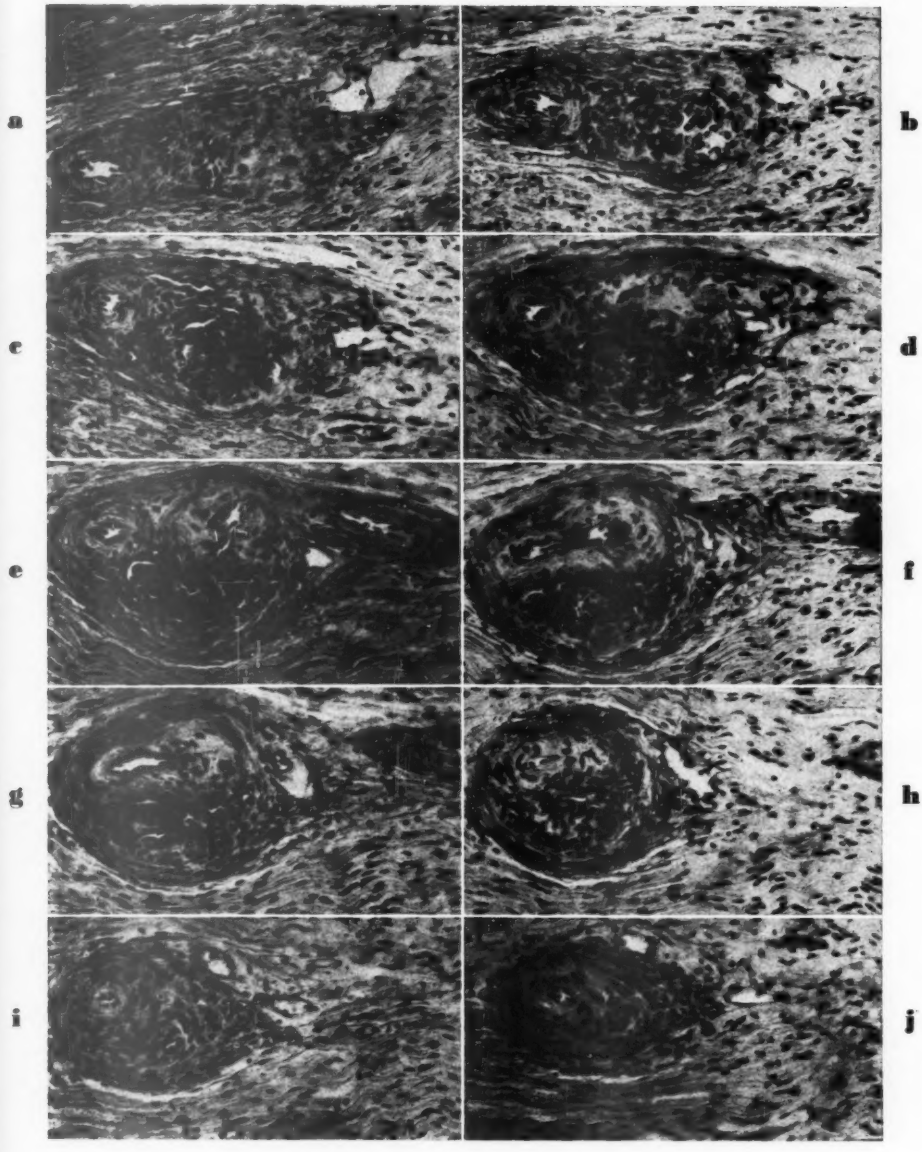



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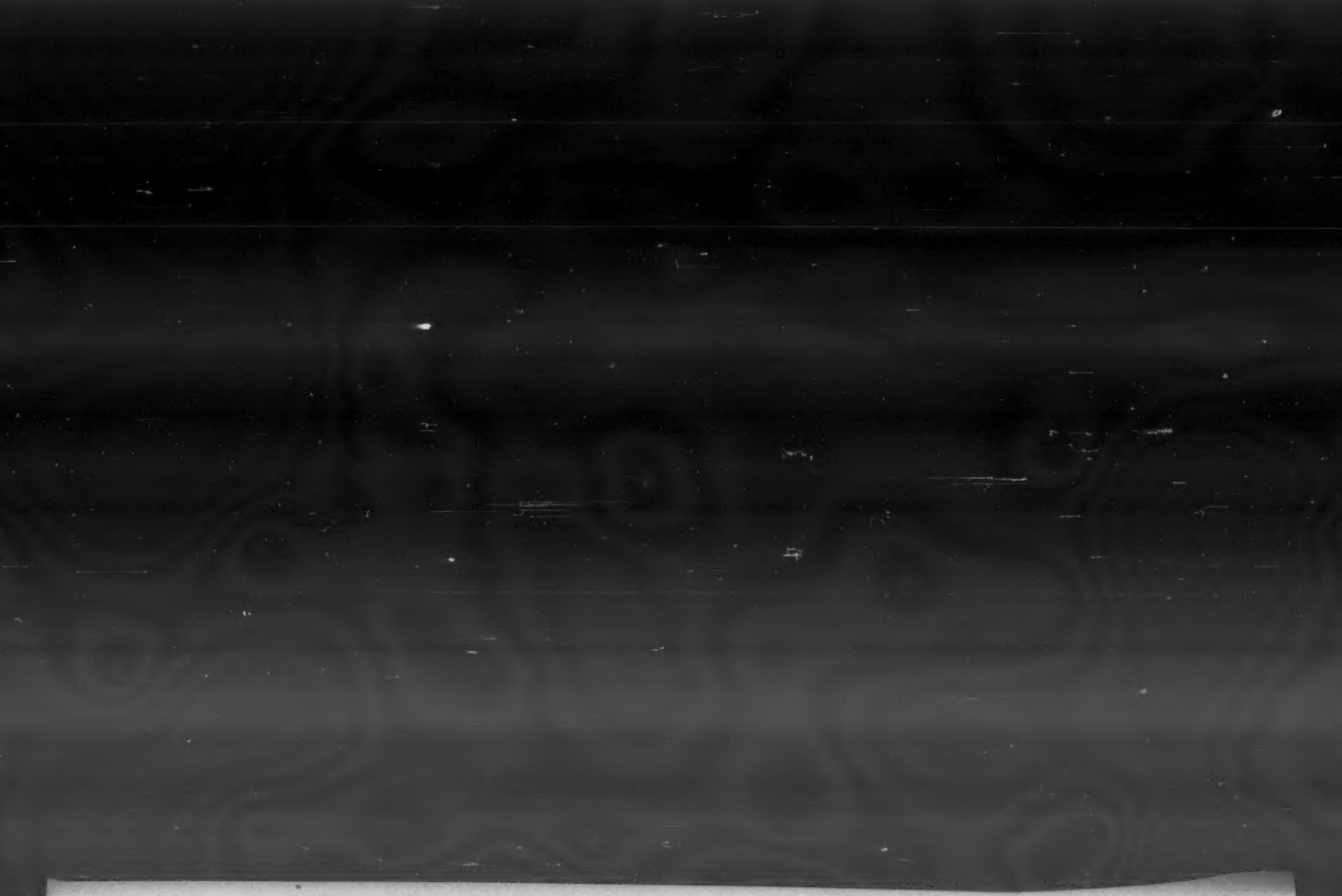
PLATE 44

FIG. 7. High-power photomicrograph of section *e* from the series shown in Figure 6. The glomerular body communicates with the lumen of the arteriole (center of field), and also with a peripheral venous sinusoid (right side of field). Hematoxylin and eosin stain. × 420.

FIG. 8. Photomicrograph of an arteriole in a sympathetic ganglion from a case of essential hypertension. The marked concentric thickening apparently is caused to a great extent by the presence of large numbers of adventitial cells. Also, in the peripheral zone there is formation of small spaces lined by flattened cells of endothelial appearance. This may be an early stage in the histogenesis of the glomerular vascular structures. Hematoxylin and eosin stain. × 650.



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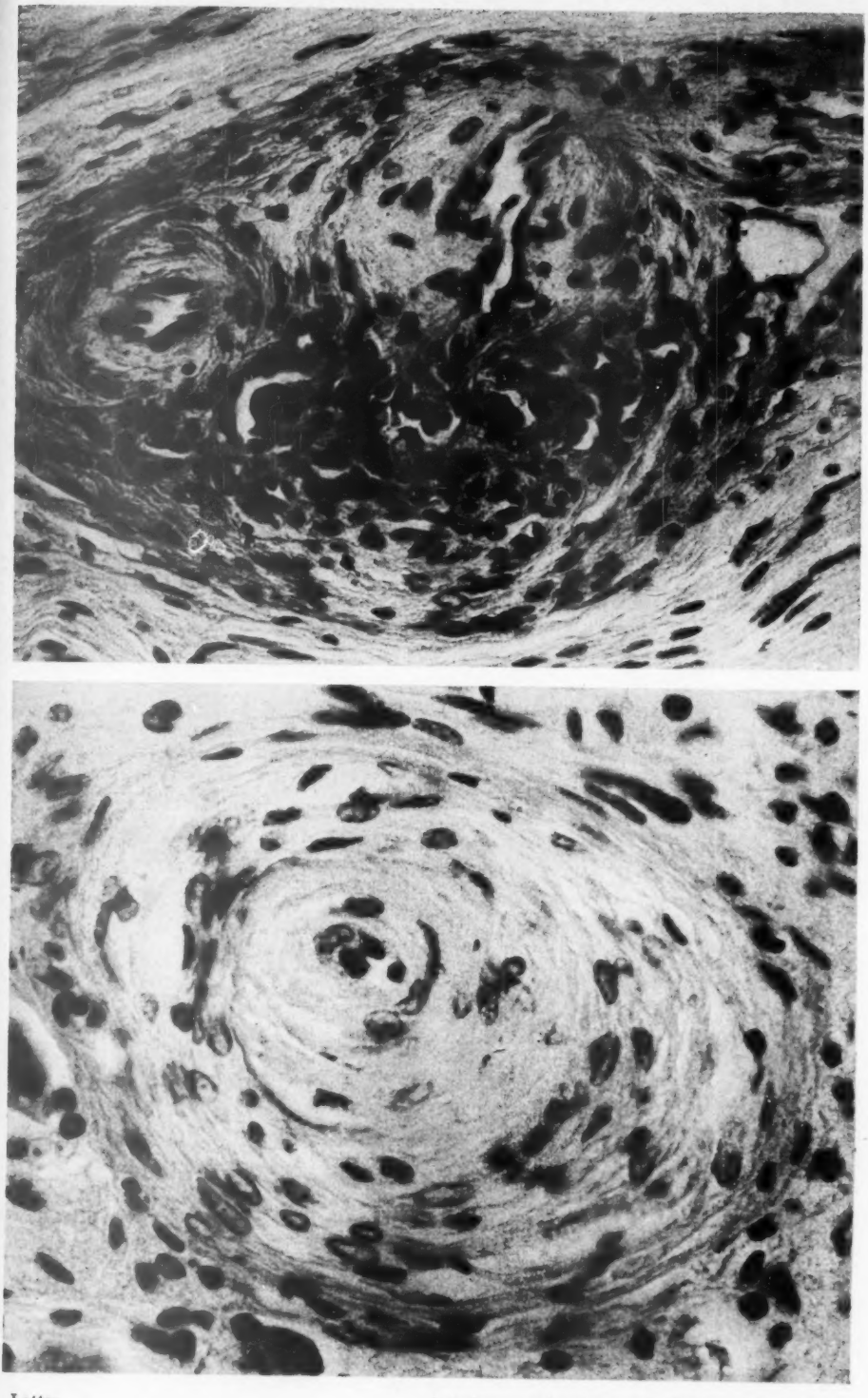

Lattes

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PLATE 45

FIG. 9. Photomicrograph showing an arteriole and smaller but similar vessels all surrounded by prominent, clear, smooth muscle cells. Between them are large, irregular, clear elements with vesicular nuclei and occasional eosinophilic cytoplasmic granules, apparently derived from the adventitia of the same vessels, and suggesting a pericytic nature. This probably represents an early stage in the histogenesis of one of the vascular glomeruli. Hematoxylin and eosin stain. × 480.

FIG. 10. Photomicrograph of a sympathetic ganglion showing a capillary blood vessel (A) emptying through a funnel-shaped channel into a large venous sinusoid (B). The other capillary blood vessel (C) running in a transverse direction is not connected with the sinusoidal space. Hematoxylin and eosin stain. × 570.

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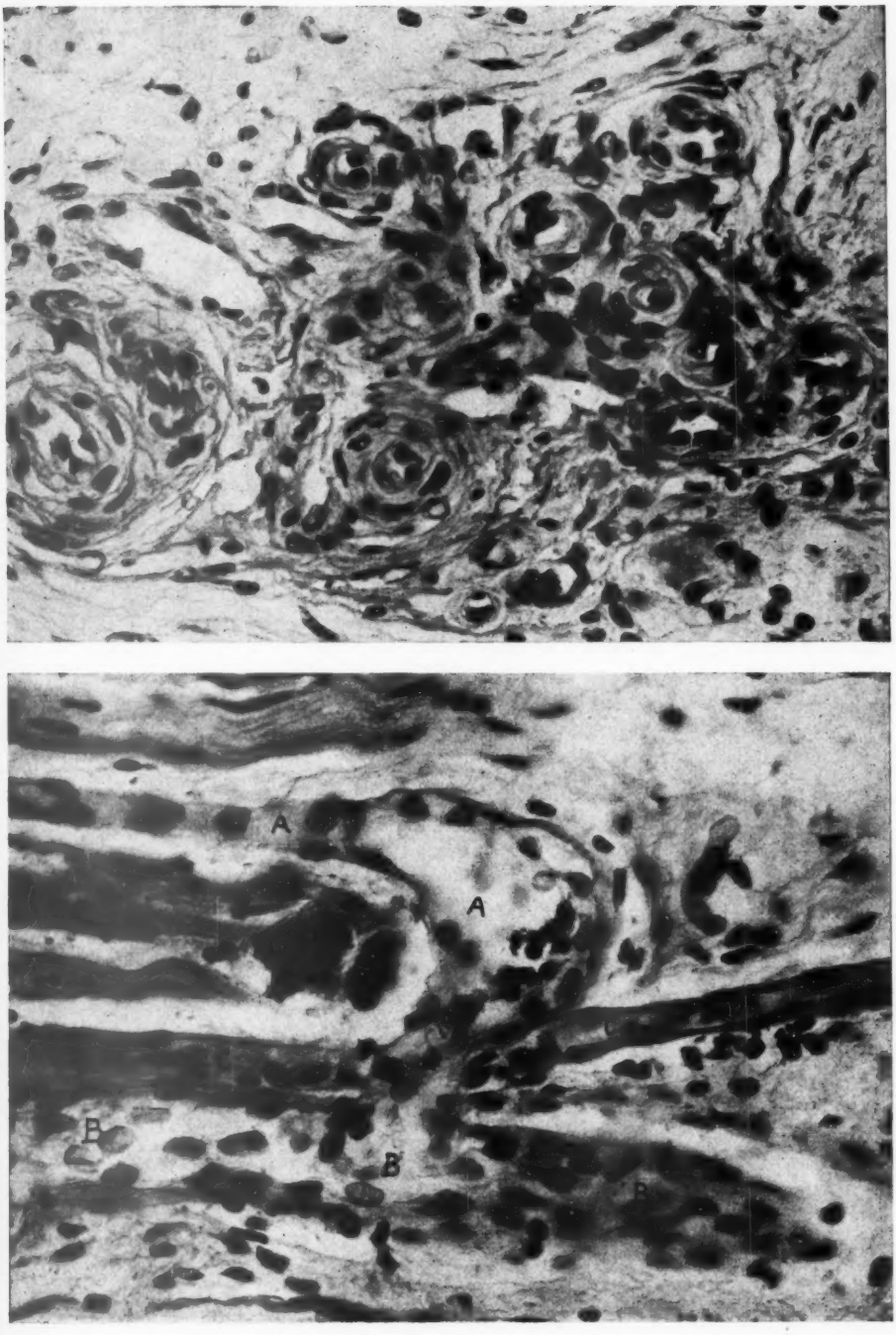


Lattes Arteriovenous Anastomoses in the Sympathetic Chain

# LESIONS IN ELASTIC ARTERIES ASSOCIATED WITH HYPERTENSION *

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It is a well established fact that morphologic alterations in the vascular system occur in hypertension, and extensive investigations have been carried out on this basis. One of the most constant of these alterations is intimal hyalinization and medial hypertrophy in the arterioles. This was described by George Johnson [1] in 1852, and designated arteriolar sclerosis. The description of this lesion by Gull and Sutton [2] established its morphologic characteristics, and the observations of Jores,[3] Bell and Clawson,[4] Fishberg,[5] and Moritz and Oldt [6] have added to the knowledge of this subject. The causal relationship between arteriolar sclerosis and hypertension, however, remains somewhat uncertain. The demonstration of the absence of arteriolar sclerosis in early cases of essential hypertension by Castleman and Smithwick [7] has been interpreted as indicating that arteriolar sclerosis is the result, rather than the cause, of hypertension.

Certain regressive and progressive changes occurring in the muscular arteries have been described from time to time in connection with the hypertensive state. Medial calcification, belonging to this group, was first observed by Virchow.[8] Mönckeberg,[9] however, gave the classical description and interpretation of this lesion, and it is now often referred to as Mönckeberg's sclerosis. Medial calcification in the arteries of the lower extremities apparently begins during childhood and increases in incidence and in severity with age. Although, as Bell [10] pointed out, hydrostatic pressure appears to contribute to its development, since the lesion is much more marked in the lower than in the upper extremities, Dietrich [11] found no definite correlation existing between medial calcification and hypertension.

In the muscular arteries, Marchand [12] and Dietrich [13] found the medial coat to have undergone hypertrophy in hypertensive subjects. In a more recent study, however, Zacharjewskaja [14] was unable to substantiate this observation in so far as the renal arteries are concerned.

Ectasia of the muscular, as well as elastic, arteries has been shown to occur in the senile period of life, and often in the absence of hypertension. Dietrich,[13] however, has shown that chronic hypertension is also a very important factor in the production of dilatation of arteries. He noted that in young and middle-aged persons, ectasia of the muscu-

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lar arteries occurred only in the presence of hypertension. Furthermore, the dilatation was more marked in senile patients if hypertension was present.

A large number of observations have been made concerning the alterations in the elastic arteries associated with hypertension. Aortic arteriosclerosis as well as that of coronary and cerebral arteries, is regularly considered to be more severe and more common in the presence of prolonged hypertension. The medionecrosis aortae idiopathica of Erdheim occurs usually in patients who are hypertensive, and, by virtue of this fact, dissecting aneurysm of the aorta is commonly associated with the pre-existing hypertension. In a careful microscopic study of the aortas in 210 routine autopsy cases, Rottino [15] noted the frequent occurrence of simple muscle loss of the media, degeneration of the elastic lamellae, and foci of cystic mucoid degeneration of the media. These alterations were slightly more common and more severe in the hypertensive than in the nonhypertensive subjects of this study.

An alteration of the elastic arteries has been noted in three hypertensive patients. Apparently this process has not been previously described.

## Report of Cases

### *Case 1*

A colored male, 55 years old, was admitted to Parkland Hospital with motor aphasia and hypertension. The extremities were cold, and light palpation over the legs was painful. Multiple soft subcutaneous nodules were distributed over the body. This patient had been in the hospital on two previous occasions: 5 years and 1 year, respectively, prior to the final admission. At the time of the first admission he had experienced a sudden onset of pain and numbness in the left arm, and axillary, radial, and ulnar pulsations were absent on that side. A scalenotomy was performed without remarkable improvement. One of the tumor masses was removed and diagnosed as "neurofibroma with myxomatous degeneration." Four years later, he was admitted complaining of headache, dizziness, and nausea. The blood pressure was 150/100 mm. Hg. The blood urea was 64 mg. per cent. The Kline test was negative and the blood urea was 16 mg. per cent. After 2 weeks of hospitalization without specific therapy of any sort, the patient's general condition had greatly improved. The clinical diagnosis was hypertensive encephalopathy. During his final admission, the patient suddenly became unconscious and exhibited hemiplegia. He expired quietly on the tenth hospital day.

At autopsy, there were numerous small subcutaneous neurofibromas over the entire body, but they were most numerous on the trunk. Focal areas of hyperpigmentation were present in the skin of the back. There were multiple, large, partially organized thrombi in the thoracic and abdominal aorta; the superior portion of the ascending thoracic aorta showed almost complete occlusion, as did the first artery arising from the arch, an arterial stem which gave rise to the two common carotid arteries. The second branch from the arch was the left subclavian artery, while the third and distal branch extended posteriorly, cours-

ing behind the esophagus, then to the right, becoming the right subclavian artery. There was a mural thrombus of the abdominal aorta, with extension into the superior mesenteric artery, and complete occlusion of the distal 8 cm. of the aorta. Thrombi also were present in both iliac and femoral arteries, in the left ventricle, in the branches of the small pulmonary artery, and in the splenic artery and vein. The left suprarenal medulla was the site of a tumor mass measuring 5.5 by 4.4 by 3.2 cm. Sections from this lesion showed it to be a paraganglioma. There was an old area of encephalomalacia in the left temporoparietal region, involving the area of the word-memory center. There were bilateral cervical ribs, and moderate arteriosclerosis of the aorta and coronary arteries.

*Case 2*

The patient was a white male, 36 years old, who had had known diabetes mellitus for 5 years, and had been admitted to the hospital many times for treatment of various infections and once with edema of the leg and hypertension. He was admitted to Parkland Hospital complaining of recurrent episodes of nausea and vomiting of 17 days' duration, and generalized edema for the same period. Examination revealed a blood pressure of 180/112, generalized edema, scarring and fresh hemorrhages in both optic fundi, and limitation of vision to light perception. Hemoglobin was 12.2 gm.; red blood cell count, 3,110,000; white blood cell count, 12,600 per cmm., with a normal differential count. The urinary findings were: specific gravity, 1.011; albumin, 3 plus; sugar, 1 plus; many red blood cells, few white blood cells, and occasional casts. Chemical studies of the blood showed: sugar, 200 mg. per cent; albumin, 2.4 gm. per cent; globulin, 2.8 gm. per cent; urea, 184 mg. per cent; cholesterol, 429 mg. per cent. The Kline test was negative. For the 22 days of his hospitalization, the patient was nauseated, continued to be edematous, and eventually became alkalotic, with $CO_2$-combining power of 81.4 volumes per cent. Tetany was treated by calcium gluconate injections. He became more edematous, and progressively drowsier, finally lapsing into coma and expiring. The clinical diagnosis was Kimmelstiel-Wilson disease.

At autopsy 5 hours after death there was marked pitting edema of feet and ankles, right leg, and abdominal and chest walls. The peritoneal cavity contained 6,000 cc. of a thin fluid, and there were 100 cc. of colorless fluid in the pericardial sac. The heart was not enlarged. Moderate arteriosclerosis of the aorta, aortic valve, and anterior mitral leaflets was noted, and there was marked coronary arteriosclerosis with narrowing of the lumina. The left ventricle showed focal areas of fibrosis. The lungs were the seat of acute passive hyperemia and edema, and showed focal patches of bronchopneumonia. The kidneys were pale, and demonstrated a smooth surface, with prominent blood vessel markings; cut section revealed a markedly pale gray structure, the cortex measuring 9 mm. in thickness and showing indistinct cortical markings. The glomeruli could easily be seen as very pale yellowish, opaque bodies. The pyramids were indistinct, and considerably enlarged. Microscopic examination showed marked diffuse fibrosis and

hyaline replacement of glomeruli, alternating with areas of structurally preserved renal tissue with dilated tubules. The interstitial tissue showed focal and diffuse lymphocytic infiltration, and there was slight to moderate arteriosclerosis and arteriolar sclerosis. The diagnosis was chronic and subacute glomerulonephritis. The duodenum showed acute mucosal erosion and small hemorrhages. There was an area of marked liquefaction in the superior portion of the left lentiform nucleus; the encephalomalacic area extended into the surrounding white substance of the cerebrum, and the internal capsule was edematous.

The orifice of the left common carotid artery was completely occluded by a moderately friable, adherent, dark reddish gray thrombus showing partial organization. This thrombus extended to the bifurcation of the common carotid artery.

*Case 3*

A white male, 60 years old, entered Parkland Hospital complaining of asthma, orthopnea, and productive cough of many years' duration. He gave a history of known hypertension for 2 years, and had had attacks of paroxysmal nocturnal dyspnea for 1 year. There had been blurring of vision and severe frontal headache for 2 months prior to admission, and for 2 weeks he had been lethargic and oliguric, and suffered from anorexia. On examination he was found to be emaciated, lethargic, and dyspneic. Temperature was 98.6° F.; blood pressure, 210/126. There was edema of the anterior chest and abdomen, along the posterior axillary folds, and over the sacrum. Dullness was elicited over the right lower lobe. The heart was not enlarged, and auscultation revealed premature auricular beats. The hemoglobin was 10 gm., and the white blood cell count was 15,400 with a shift to the left in the Schilling hemogram. The specific gravity of the urine was 1.012 and the albumin was 4 plus. Chemical studies of the blood showed: urea, 105 mg. per cent; total serum proteins, 7.3 gm. per cent; albumin, 3.3 gm. per cent; globulin, 4.0 gm. per cent; $CO_2$-combining power, 53.1 volumes per cent. Venous pressure was 65 mm. of water, and the circulation time was 23 seconds with decholin. The Kline test was negative. The patient received digitalis and aminophylline, and seemed to be improving, when he quietly expired on the eighth hospital day. The clinical diagnoses were "chronic glomerulonephritis, bronchial asthma, pulmonary emphysema, and generalized arteriosclerosis."

At autopsy, the peritoneal cavity contained 5,500 cc. of colorless fluid. The aorta showed ulcerated and hemorrhagic atherosclerotic plaques. There was moderate left ventricular enlargement of the heart, the left wall measuring 2 cm. in thickness. There was marked coronary atherosclerosis. The lungs showed acute passive hyperemia, slight edema, and chronic fibrous pleuritis on the right. The kidneys were pale and presented a finely granular surface with many uniformly distributed areas of indentation. The cortical markings were not prominent. Microscopic study showed a marked degree of arteriolar nephrosclerosis.

In the gross examination of the elastic arteries of these three cases, thrombosis of the aorta and other arteries in case 1 and of the left

common carotid artery of case 2 is the outstanding observation. Atherosclerosis was evident in some degree in each case; however, no further alterations were noted. A composite description of the microscopic changes in the elastic arteries of these three cases follows.

### Histologic Observations on Involved Arteries

The principal alteration in the elastic arteries was in the medial coat. The outer musculo-elastic layers appeared to be partially collapsed, so that individual elastic lamellae lay close together and were for the most part intact. Some elastic fibers were broken into small, separate, refractile particles and were undergoing disintegration. This outer portion of the medial coat was the seat of marked increase in cellularity, due to proliferation and exudation. Elongated, fixed cells resembling fibroblasts were the proliferating constituents and these were regularly arranged parallel with the lamellae of the medial coat. The nuclei of these cells were hypertrophied and pale. Many new capillaries also were present in the area, extending in from the adjacent adventitia. Many of these vessels were represented by small, solid cords of endothelial cells without lumina while others possessed small lumina. The endothelial cells, like the fibroblasts, had swollen, hypochromatic or vesicular nuclei. Besides the young capillary units, there also were numerous thin-walled vessels, predominantly veins, which extended into the media from the adventitia. Some of these were arranged at right angles, while others extended circularly around the vessel. The degree of proliferation and vascularization was marked in cases 1 and 2, while in case 3 it was slight.

The exudative constituent of the medial lesion consisted of moderate numbers of lymphocytes which were diffusely scattered throughout the outer medial coat, being most numerous in the regions of capillaries and where proliferative changes were most pronounced. Occasional polymorphonuclear cells and large mononuclear wandering cells were present also. In case 3, in which there was marked fragmentation of the musculo-elastic layer and small foci of coagulative necrosis in the outermost portion of the media, polymorphonuclear cells were the main exudative component.

In the adventitia there was a slight to moderate infiltration of lymphocytes, which were primarily collected in the region of blood vessels. These cellular infiltrates differed in appearance from those seen in syphilitic aortitis in their failure to form complete and intimate perivascular collars. The infiltration did not involve the vasa vasorum, and these vessels were not the seat of endarteritis. In the portion of the adventitia adjacent to the media, the collagenous bundles were

thickened and there was fibrous tissue proliferation, leading to slight cicatrization. Collagen bundles near the media were found to be markedly fragmented and occasionally the seat of early coagulative or fibrinoid necrosis. Capillaries with swollen endothelial cells were noted in this portion of the adventitia.

The vasa vasorum were found to have thickened walls so that the lumen/wall ratio was markedly reduced. There was a slight degree of hyaline deposit in the intima, but the most striking change was thickening of the muscle coat of the vasa vasorum. The muscle fibers of the media were distinctly increased in number, so that several layers of muscle cells had developed in these vessels. The individual muscle fibers were hypertrophied and their nuclei swollen.

In each of the cases reported there was atherosclerosis, involving the intima. In cases 1 and 2 atherosclerosis was slight or moderate. In case 3 it was marked, but in this instance the lesions described above were found in portions of the aorta where atherosclerosis was slight.

### Histologic Study of Aorta and Other Elastic Arteries in Hypertensive and Nonhypertensive Patients

Microscopic study of the aorta, and in many cases also of the innominate, left common carotid, and left subclavian arteries, was made in 40 patients who had hypertension. Thirty-one of this group had essential hypertension, 5 had pyelonephritis, 3 had glomerulonephritis, and one had hypertension due to a paraganglioma of the adrenal.

Atherosclerosis was present in at least a mild degree in every case, and in general it was considerably more marked in the hypertensive group than in the control group of nonhypertensive patients. In 15 patients atherosclerosis of the elastic arteries was the only alteration present on gross and microscopic examination. Two patients had syphilitic aortitis. The remaining 23 cases revealed some degree of histologic alteration of the aorta which was considered to be distinct from the effects of atherosclerosis. The histologic changes were marked in 6 of these, moderate in 11, and slight in 6. Two patients in this group had arteriosclerotic aneurysms of the abdominal aorta.

The histologic changes referred to were located in the media and adventitia and also involved the vasa vasorum. The most common alteration consisted of small collections of lymphocytes in the adventitia. Except in those cases in which syphilitic aortitis was present, no fibrous proliferation was encountered. In the media, increase in number and in prominence of capillaries and other small vessels were very common observations. The endothelial cells lining these small vessels often were swollen. Small numbers of lymphocytes and polymorphonu-

clear leukocytes frequently were seen around these vessels in the media. Occasionally, small foci of degeneration or necrosis were noted in the outer portion of the media, associated with disruption of the medial elastic and muscular lamellae. Less common observations relating to changes in the media were atrophy of the musculo-elastic lamellae with reduction in thickness of the layer, areas of limited fibrous replacement, and mucoid degeneration.

In the vasa vasorum, hypertrophy of the muscle coat and, occasionally, subendothelial hyaline deposition were commonly noted. The incidence of this arteriolar sclerosis was 35 per cent.

It will be seen that these alterations occurring in the aortas of a series of hypertensive patients are similar, except for their milder form, to those described in the three reported cases.

In a group of 50 control cases without hypertension, microscopic examination of the elastic arteries failed to reveal alterations approaching those which have just been described. Atherosclerosis of marked degree was present in numerous cases and occasionally in such cases, small collections of lymphoid cells were noted in the adventitia. Hypertrophy and hyaline deposition were not found in the vasa vasorum in any case. Atrophy of the media associated with marked atherosclerosis was noted occasionally. Otherwise medial changes were not encountered.

Analysis of the clinical and pathologic data in the hypertensive series showed that there was no correlation between the incidence or the severity of the changes in the aorta, and the age or sex of the patients, uremia, the level of blood pressure, the etiologic mechanism of the hypertension, or serologic and pathologic evidence of syphilis.

On the other hand, there was noted a certain degree of correlation between the severity of the lesions of the medial and adventitial coats of the aorta and the severity of the atherosclerosis. The degree of correlation is not great, and there were several cases of marked medial and adventitial alterations with only slight atherosclerosis. In a considerable number of cases of marked atherosclerosis medial and adventitial changes were slight or absent.

## Comment on Pathogenesis and Significance of Arterial Lesions

The lesions described here bear some similarity to periarteritis nodosa. Especially since recent studies by Cromartie,[16] and Loomis [17] have demonstrated the occurrence of periarteritis in rats which had been rendered hypertensive, the possibility of so designating the lesions described here must be considered. The distribution of the arterial lesions in the cases reported here is not that of periarteritis as seen

clinically, nor is it that of the type of periarteritis observed in hypertensive rats. In Loomis' study, the mesenteric arteries were primarily involved, although lesions in the elastic arteries were recorded and described. The principal involvement of the media, the presence of degenerative changes in the elastic fibers, the muscular hypertrophy of vasa vasorum, and the absence of eosinophils in the lesions as described, indicate that fundamental differences from periarteritis nodosa exist. None of the three patients reported here had received sulfonamide drugs, although periarteritis certainly occurs in the absence of such medication.

From the morphologic standpoint it appears relatively certain that this arterial lesion is not due to syphilis. The absence of typical perivascular lymphoid collars in the adventitia, of endarteritis of the vasa vasorum, of characteristic meso-arteritis, and of gummatous necrosis of the media are the main points of difference between the arterial lesion described and that of syphilis. Serologic tests for syphilis were negative in the reported cases and no other visceral lesions of syphilis were found. Likewise, the occurrence of less severe aortic lesions of the same type were noted in the series of hypertensive cases in which no correlation with serologic or pathologic evidence of syphilis could be demonstrated.

Observations on medial changes of the elastic arteries in hypertension must take into account the lesion described by Erdheim,[18] named by him "medionecrosis aortae idiopathica," and since studied by Moritz[19] and by Rottino.[20] This lesion, as pointed out by various observers, is a cystic necrosis developing in the central portion of the media, and absence of proliferative and exudative reaction is characteristic of the process. In this respect there is a fundamental difference between this type of medial necrosis and the lesion reported and described here.

There have been several reports[21,22] of experimental studies which indicated the occurrence of changes in arteries due to the administration of adrenalin and other vasoconstrictor agents. These authors have reported degenerative changes, necrosis, and secondary inflammatory reaction in the media and adventitia of the aorta and of other vessels throughout the body. The changes described are similar to those reported here in human subjects with hypertension. In view of the muscular hypertrophy of the vasa vasorum of the vessels involved in our cases, and in view of the vasoconstrictive state which is understood to accompany the hypertensive process, we may assume the pathogenesis of the lesions described here to originate with constriction of the vasa vasorum. This vasoconstriction would necessarily lead to anoxia

of the arterial wall, which, if severe enough, would produce degenerative changes, necrosis, and secondary inflammatory reaction in the vessel wall. In case 1, as reported, the mechanism of constriction of the vasa vasorum is apparently the excessive output of adrenalin by the paraganglioma. In the other cases, one of hypertension associated with chronic glomerulonephritis and the other of essential hypertension, the constriction of the vasa vasorum might be attributed to the general vasoconstrictive mechanism in these cases. The degenerative and inflammatory arterial lesions would be expected to occur more frequently and in more severe form in those cases in which the hypertension is of a severe grade.

One may consider what the primary event is when the medial lesions here described are found accompanying atherosclerosis. In syphilis of the aorta it is well known that the changes in the outer layers of the aorta predispose to atherosclerosis. Although it is quite certain that intimal lipoid deposition and hyalinization may occur in the presence of an otherwise entirely normal vessel wall, nevertheless it seems likely that the intimal deposits are hastened and exaggerated by medial disease. This explanation might account in part for the exaggerated atherosclerosis in the aortas of patients with hypertension, since degenerative and inflammatory medial lesions seem to occur in this group. However, increased pressure within the lumina of the elastic arteries appears to be a factor of great importance in the development of atherosclerosis in hypertensive patients.

The occurrence of marked thrombosis of the large arteries which was observed in two of the reported cases is of considerable importance. In case 1, this phenomenon was at least contributory to the production of death, if not the main factor therein. Atherosclerosis, which was present in mild to moderate degree in these cases, cannot be considered the causative factor of the thrombosis since very severe and "ulcerative" atherosclerosis of the aorta produces only very small thrombi over the ulcerated plaques. Furthermore, the atherosclerosis in the two cases with thrombosis was not extreme, nor was it ulcerative. We are led to believe that the same vasoconstrictive anoxia, which is thought to account for the medial and adventitial injury, likewise produced an extensive intimal endothelial injury followed by thrombosis.

It is tenable that the vasoconstrictive anoxia developing in this manner and present over a long period, produces the type of intimal injury which may be followed by atherosclerosis, according to the anoxic theory of atherosclerosis recently detailed by Hueper.[23] On the basis of this hypothesis, it is possible to account for the development of both medial lesions and atherosclerosis in hypertensive disease, and to ac-

count for the correlation in severity of these two apparently separate lesions that has been shown to exist in this report.


## Summary

Three cases are reported in which unusual lesions were noted in the aorta and large elastic arteries accompanying hypertension. In two cases thrombi were present in the involved arteries. This alteration of the elastic arteries involved the media most strikingly. In its outer portion there were degeneration, necrosis, and collapse of the musculoelastic lamellae, accompanied by an exudation of lymphocytes and other inflammatory cells, and by proliferation of fibroblasts and capillary endothelial cells. In the adventitial layer there were perivascular lymphocytic infiltration, and muscular hypertrophy and hyperplasia with subendothelial hyaline deposition of the arteriae vasorum.

In a review of 40 hypertensive cases, similar but much less marked alterations of the aorta and/or other large elastic arteries were noted in 23.

On the basis of histologic findings, serologic tests, and other accompanying pathologic and clinical data, it is considered that the lesions of elastic arteries described are separate and distinct from atherosclerosis, syphilitic arteritis, periarteritis nodosa, and medionecrosis of the aorta. It is suggested that constriction of the vasa vasorum associated with the hypertensive state might lead to ischemia with injury of medial and adventitial layers resulting in the degenerative, inflammatory, and proliferative alterations noted, and that thrombosis might be explained on the basis of ischemic intimal injury.


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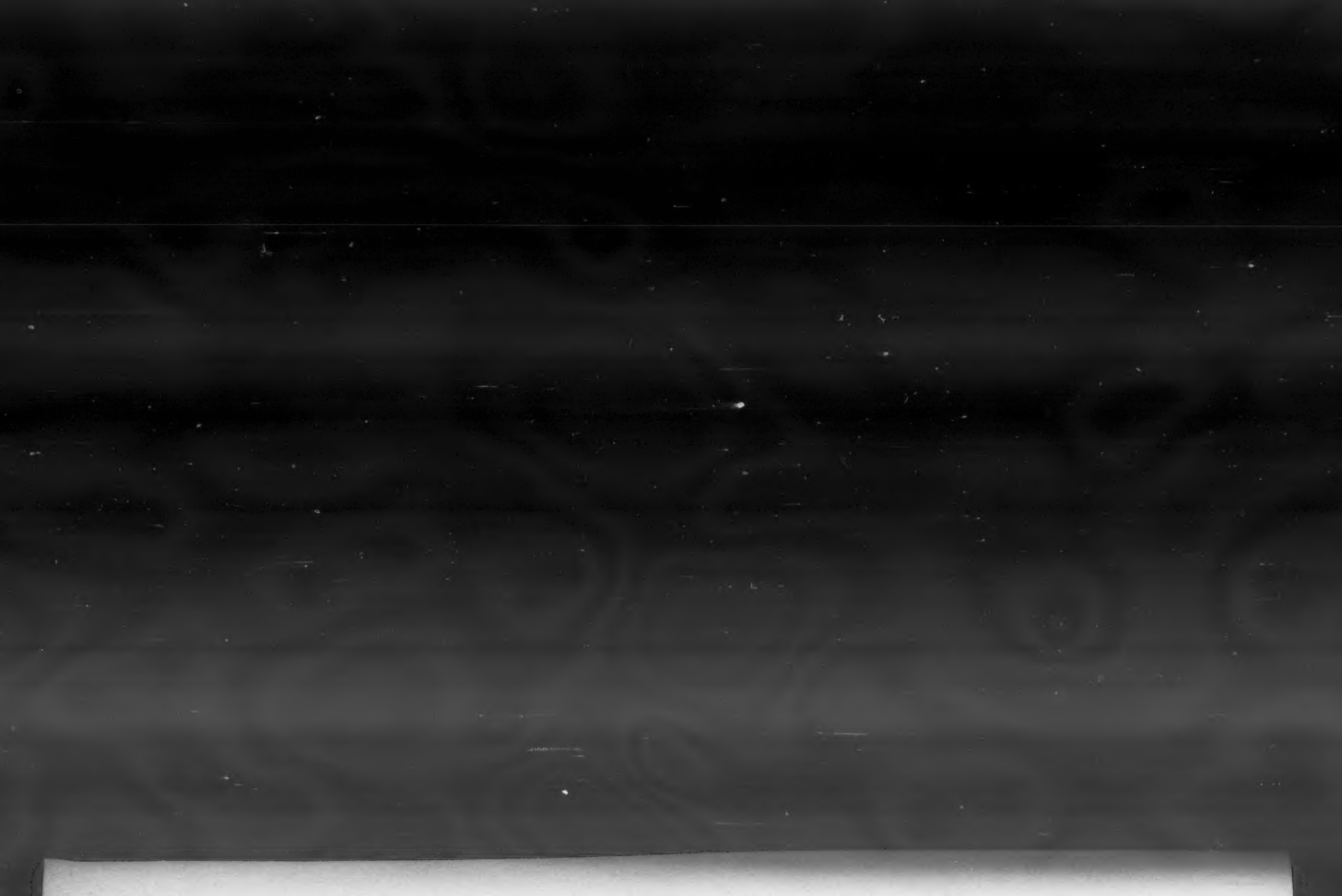
[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

### PLATE 46

FIG. 1. Photomicrograph of the aorta in case 1. A portion of the thrombus is adherent to the intima. Slight atherosclerosis is present. The outer medial coat shows marked exudative and degenerative changes. Scarring and lymphoid infiltration are present in the adventitia. Hematoxylin and eosin stain. × 130.



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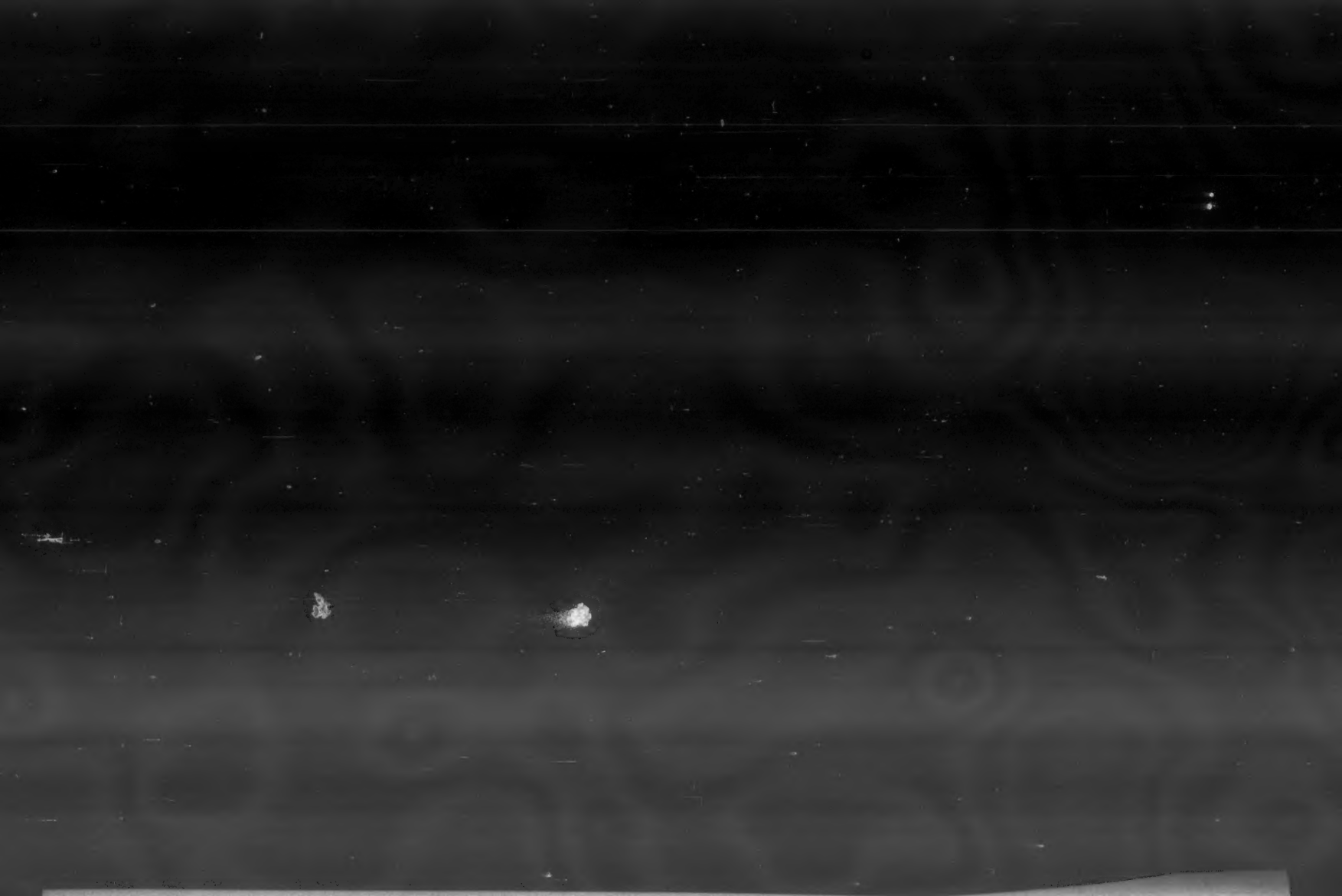
Ashworth and Haynes Lesions in Elastic Arteries

PLATE 47

FIG. 2. Photomicrograph of the left common carotid artery in case 2. An organized thrombus occupies the lumen. The outer medial coat and adventitia are infiltrated with lymphocytes. Of note are the degeneration and necrosis of the medial musculo-elastic lamellae. The arteriae vasorum in the adventitia reveal medial hypertrophy. Hematoxylin and eosin stain. × 175.



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Ashworth and Haynes Lesions in Elastic Arteries

# ALTERATIONS OF CEREBRAL CAPILLARIES IN THE EARLY STAGE OF ARTERIAL HYPERTENSION *

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The paramount significance of arterial hypertension in the production of cerebral tissue alterations has been emphasized repeatedly in recent studies.[1-4] The object of this paper is to analyze the *earliest* stage of cerebral vascular changes in cases of arterial hypertension, with special emphasis upon those seen in the cerebral capillaries. This study is based upon 6 fatal cases of arterial hypertension in which death occurred from 1 to 2 years after the onset of the disease. No attempt will be made to describe the findings in each case. Two illustrative cases, however, are presented in detail.

## Report of Cases

### CASE 1

A white male, 50 years of age, was admitted to the hospital with a history of arterial hypertension of 1 year's duration. He had been in good health until 2 weeks prior to admission, at which time he noticed severe frontal and occipital headache, malaise, and shortness of breath. During the last few days, the patient had had several bouts of paroxysmal nocturnal dyspnea. His wife stated that, within the past 2 weeks, the patient had shown occasional signs of confusion, disorientation, and forgetfulness.

*Examination.* Upon admission the patient was extremely restless and confused. His temperature was 100° F.; pulse, 94; respirations, 20; blood pressure, 230/140 mm. Hg. There was a suggestion of bilateral exophthalmus. The pupils were 4 mm. wide, and reacted normally to light and in accommodation. Examination of the fundi disclosed bilateral papilledema and a few hemorrhages. The neurologic findings were normal.

*Course.* The initial state of confusion progressed rapidly through delirium to stupor and coma. The patient finally developed generalized convulsions, and died on the ninth hospital day.

*Laboratory Data.* A lumbar puncture performed on the third hospital day yielded clear, colorless cerebrospinal fluid under pressure of 600 mm. of water; it contained no cells; the total protein content was 138 mg. per 100 cc., and the Wassermann reaction was negative. A spinal tap performed on the seventh hospital day yielded xanthochromic fluid under pressure of 210 mm. of water; it contained no cells. The urine was grossly bloody and contained granular casts and albumin. The blood urea nitrogen on the second day after admission was 130 mg. per 100 cc. and subsequently (3 days before death) rose to 210 mg. per 100 cc.

### *Necropsy Findings*

The pathologic alterations, exclusive of those found in the brain, were summarized as bilateral confluent lobular pneumonia; cardiac

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hypertrophy and dilatation; arteriolonephrosclerosis; right pleural and pericardial fibrosis; and petechial hemorrhages in the pleura, kidneys, pelvis, and urethra.

Examination of the nervous system was limited to the brain, which weighed 1400 gm. and showed a generalized increase in bulk. The gyri were flattened and the sulci narrowed. There was a slight increase in subarachnoid fluid in the parietal region near the midline. The pons, medulla, and cerebellum were not remarkable. There was a well defined cerebellar pressure cone. The blood vessels of the circle of Willis were normal in distribution and showed changes characteristic of arteriosclerosis.

Coronal sections through both hemispheres revealed a diffuse swelling of the white matter of the left hemisphere, with consequent compression of the left lateral ventricle. In the right hemisphere there was a massive hemorrhage in the vicinity of the island of Reil and lateral to the internal capsule. Sections of the brain stem disclosed a small ball hemorrhage on the left side of the pons. In addition there was a diffuse swelling of the midbrain, with loss of demarcation between gray and white matter. Sections of the medulla and cerebellum revealed no gross abnormalities.

## Microscopic Findings

Histologic examination disclosed abnormalities of two principal types: (1) vascular alterations, and (2) changes in the nervous tissue.

The chief histologic alterations of the vascular system are shown in Figures 1 and 2. The vascular changes were confined essentially to the capillaries. These exhibited a uniform pathologic process consisting of a combination of extreme cellularity and degenerative alterations of the vessel walls. The increased cellularity of the capillaries was the most striking and the most widespread vascular alteration. It was caused chiefly by an extreme degree of fibroblastic proliferation of the adventitial membrane and a pronounced hyperplasia of the lining endothelium. In addition there was a pericapillary accumulation of glial nuclei, resulting from the marked glial hyperplasia and hypertrophy of the surrounding nervous tissue. Only occasionally were there seen a small number of macrophages and a few lymphocytes.

The degenerative alterations consisted of homogenization and thickening of the vessel walls with resultant narrowing or complete obliteration of the vascular lumina. In many instances the entire capillary was converted into a structureless, homogeneously stained, hyalinized, solid nodule composed of a central homogeneous mass surrounded by a ring of proliferated fibroblasts and glial cells (Fig. 3). The central portion obviously represented the remnants of the completely degenerated and hyalinized capillary, the lumen of which was obliterated.

The arterioles exhibited only a moderate degree of thickening and hyalinization of their walls. Changes characteristic of advanced hypertensive arteriolopathy were seen only occasionally.

More conspicuous were the changes of the cerebral veins. In addition to the massive hemorrhage involving the right basal ganglia, there were a few so-called ball hemorrhages in the form of small, sharply demarcated hemorrhagic foci measuring from 5 to 50 mm. in diameter. They were seen in areas quite remote from the massive hemorrhage, and consisted of one or several central veins surrounded by large masses of extravasated blood. The central blood vessels were mostly medium-sized, extremely congested veins; their lumina were distended, and far advanced degenerative changes had occurred in their walls.

The degree of degeneration of some of the veins is illustrated in Figure 4. As here shown, the vascular wall had undergone almost complete disintegration and necrosis. The normal appearance of the division into three coats was completely lost. The entire vessel wall was transformed into a structureless, homogeneous substance devoid of either muscular or elastic elements. Its outer margin was bounded by a thin connective tissue membrane with a small number of nuclei which were barely visible. In some instances, the perivascular space of the vein was distended and harbored red blood cells and macrophages.

Changes of the nervous tissue included both diffuse alterations of the parenchyma and vascular alterations characteristic of cerebral swelling.[5] Figure 5 illustrates the characteristic spongy appearance of the central white matter caused by the tremendous distention of the pericellular and perivascular spaces and by numerous large, oval spaces filled with serous fluid. The latter contained neither cellular elements nor stainable substance. Numerous nerve fibers and myelin sheaths displayed various stages of degeneration, such as irregularity of contour, beading, or complete loss of stainability.

The general vascular alterations consisted of congestion, stasis, and morphologic signs of vasoparalysis of the smaller veins and capillaries. In many smaller blood vessels there were various degrees of degeneration of the walls, associated with increased permeability for serous fluid which had transuded into the distended perivascular spaces. These changes were most pronounced within the swollen portions of the white matter.

### CASE 2

A white female, 41 years of age, was admitted to the hospital with a history of hypertension of approximately 2 years' duration. She was first admitted in November, 1945, because of attacks of asthma associated with purulent bronchitis and fever. On routine examination hypertension of 205/140 mm. Hg was found. There had been no symptoms related to high blood pressure except occasional headaches.

*Physical examination* disclosed the following pertinent findings: The optic disks were mildly congested, but there was no definite papilledema. The retinal arterioles showed attenuation and areas of localized spasm, with compression at venous crossings; there were multiple cotton-wool patches of exudate and perivascular flame-shaped hemorrhages in both eyes. The neurologic examination disclosed normal findings.

*Laboratory Findings.* Concentration tests of urine showed a variation from 1.006 to 1.016 in specific gravity. Repeated urinalyses for sugar and albumin were negative; only on one occasion was 1 plus albumin found. The sediment contained only a few white blood cells and was otherwise negative. The blood urea nitrogen was 24, 23, and 17 mg. per 100 cc. Other studies, including phenolsulfonphthalein test and pyelography, were normal. Electrocardiographic examination of the heart yielded normal findings.

*Second Hospital Admission.* On February 18, 1946, the patient was admitted for the second time. For the past 3 months she had been kept on a low sodium diet. Her chief complaints were orthopnea, dyspnea, and swelling of the ankles. Physical examination revealed signs of right-sided cardiac failure with hepatic congestion. The neck veins were distended; the venous pressure was 24 cm. of water. The hepato-jugular reflex was positive.

*Clinical Course.* During the second hospital week the patient began to complain of visual disturbances. Examination of the fundi disclosed papilledema, more pronounced in the right eye than in the left. The retinal veins were distended; no fresh hemorrhages were seen.

The patient frequently complained of nocturnal respiratory distress. During her hospital stay she had several attacks of asthma. The blood pressure varied between 194/130 and 210/140.

On March 21, 1946, a left Smithwick procedure was performed. The patient tolerated the operation quite well, and the postoperative course was uneventful. Her blood pressure remained essentially at the same level, varying between 210/130 and 190/140. Following the operation she complained of complete loss of vision in the right eye. Ophthalmoscopic examination disclosed extensive edema of the retina and of the optic disk on the right. On April 1, 1946, a right Smithwick procedure was performed. At the end of the operation, as the skin was being closed, the patient ceased to breathe and could not be revived.

## *Necropsy Findings*

The pathologic changes, exclusive of those found in the brain, were summarized as acute catarrhal bronchitis with focal areas of pulmonary atelectasis; focal lobular pneumonia of the right lower lobe; right pneumothorax; accelerated arteriolonephrosclerosis; coronary, pulmonic, and aortic atherosclerosis; acute passive hyperemia of the viscera; mural thrombosis of the right atrial appendage; small focal areas of degeneration in the myocardium.

Examination of the nervous system was limited to the brain. On gross examination it appeared normal. The major blood vessels were thin-walled and of somewhat translucent appearance; small sclerotic plaques were seen only occasionally. Coronal sections through both hemispheres showed, except for a mild degree of congestion, normal findings. Sections through the midbrain revealed diffuse swelling of the tegmen of the pons, with almost complete obliteration of the lower

aqueduct and of the lumen of the fourth ventricle. In addition, there were a few small hemorrhages in the periaqueductal region.

### Microscopic Findings

The most striking histologic findings were confined to the capillaries. These exhibited uniform pathologic lesions similar to those described in case 1. Figure 6 illustrates the increased cellularity of the capillary wall, caused chiefly by fibroblastic proliferation of the adventitial membrane and by hyperplasia and hypertrophy of the lining endothelium. The surrounding nervous tissue reacted, as a rule, with a focal area of pericapillary glial proliferation. In some instances, however, degenerative alterations of the capillaries were prevalent. These consisted of homogenization and thickening of the vessel wall, with resultant narrowing or complete obliteration of the vascular lumen (Fig. 7).

The arterioles were relatively slightly affected. Only occasionally were there changes characteristic of advanced hypertensive arteriolopathy (Fig. 8).

Changes of the nervous tissue consisted of areas of rarefaction with early signs of necrosis. The nerve fibers and nerve cells showed loss of stainability and signs of ischemic degeneration. In addition there were a few perivascular hemorrhages in the periaqueductal region and in areas adjacent to the fourth ventricle.

### *Summary of Pathologic Findings*

In previous contributions [1,2] attention was drawn to vascular alterations in hypertensive encephalopathy. These histologic changes, which were regarded as typical, consisted of hyaline degeneration and fibrotic thickening of the vessel walls with narrowing or complete obliteration of the vascular lumina. These vascular changes, confined to arterioles and capillaries, are different from those found in arteriosclerosis and are to be interpreted as a special form of hypertensive arteriolopathy. The alterations of the nervous parenchyma characterized by diffusely scattered small foci of old and recent softening were interpreted as secondary to the vascular lesions.

In addition to the arteriolar changes, two types of *venous* alterations were described recently [3,4]: (a) reversible changes characterized by stasis, congestion, and distention of the vascular lumina; and (b) structural lesions of the vessel walls manifested by advanced signs of degeneration, necrosis, and/or an extreme degree of atrophy. Whereas the arteriolar changes were interpreted as significant for the diffuse focal areas of softening and gliosis of the nervous parenchyma typical

of hypertensive encephalopathy, the alterations of the cerebral veins were regarded as responsible for the origin and pathogenesis of the massive intracerebral hemorrhages so often encountered during the terminal phase of hypertensive brain disease.

In all cases in the present study the predominant pathologic alterations were confined to the capillaries. The vascular lesions were characterized by a combination of proliferative and degenerative changes of the capillary walls. The proliferative alterations were the result of fibroblastic proliferation of the adventitial membrane associated with pronounced hyperplasia and hypertrophy of the lining endothelium. The degenerative changes consisted of homogenization and thickening of the capillary wall with resultant narrowing or complete obliteration of the vascular lumen. In many instances the entire capillary was converted into a structureless, homogeneously stained, hyalinized solid nodule composed of a central homogeneous mass surrounded by a ring of proliferating glial cells and fibroblasts. The central portion obviously represented the remnants of the completely degenerated and hyalinized capillary, the lumen of which was obliterated.

In all cases under observation, death occurred from 1 to 2 years after onset of the clinical manifestations of the disease. It is concluded, therefore, that the afore-described capillary changes may be considered as the early stage of hypertensive brain disease.

Of special interest was the attempt to correlate the findings in the central nervous system with those in the kidneys. No direct parallelism could be established. Whereas in some cases the kidneys disclosed changes characteristic of accelerated nephrosclerosis, in others they displayed minimal changes or a moderate degree of an arteriosclerotic vascular process.

It is noteworthy that case 1 of the present study exemplifies a characteristic clinicopathologic syndrome of arterial hypertension recently described under the heading of "hypertensive cerebral swelling." [6] The clinical picture is characterized by sudden onset and rapid progression of severe headache, drowsiness, confusion, restlessness, and delirium, accompanied by signs of increased intracranial pressure, such as elevation of spinal fluid pressure and papilledema. The underlying histopathologic findings consist in various stages of cerebral swelling.

Case 1 of this study revealed, in addition to cerebral swelling, a massive intracerebral hemorrhage and vascular alterations (venous and arteriolar changes) characteristic of hypertensive brain disease.

## Summary

Attention is directed to an early stage of cerebral vascular change in cases of arterial hypertension, in which the vascular alterations are

confined to the capillaries, and are characterized by a combination of proliferative and degenerative changes.

In all cases of the present study, death occurred from 1 to 2 years after onset of the clinical manifestations of the disease.

No direct parallelism between the cerebral vascular changes and those of the kidneys could be established.

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[*Illustrations follow*]

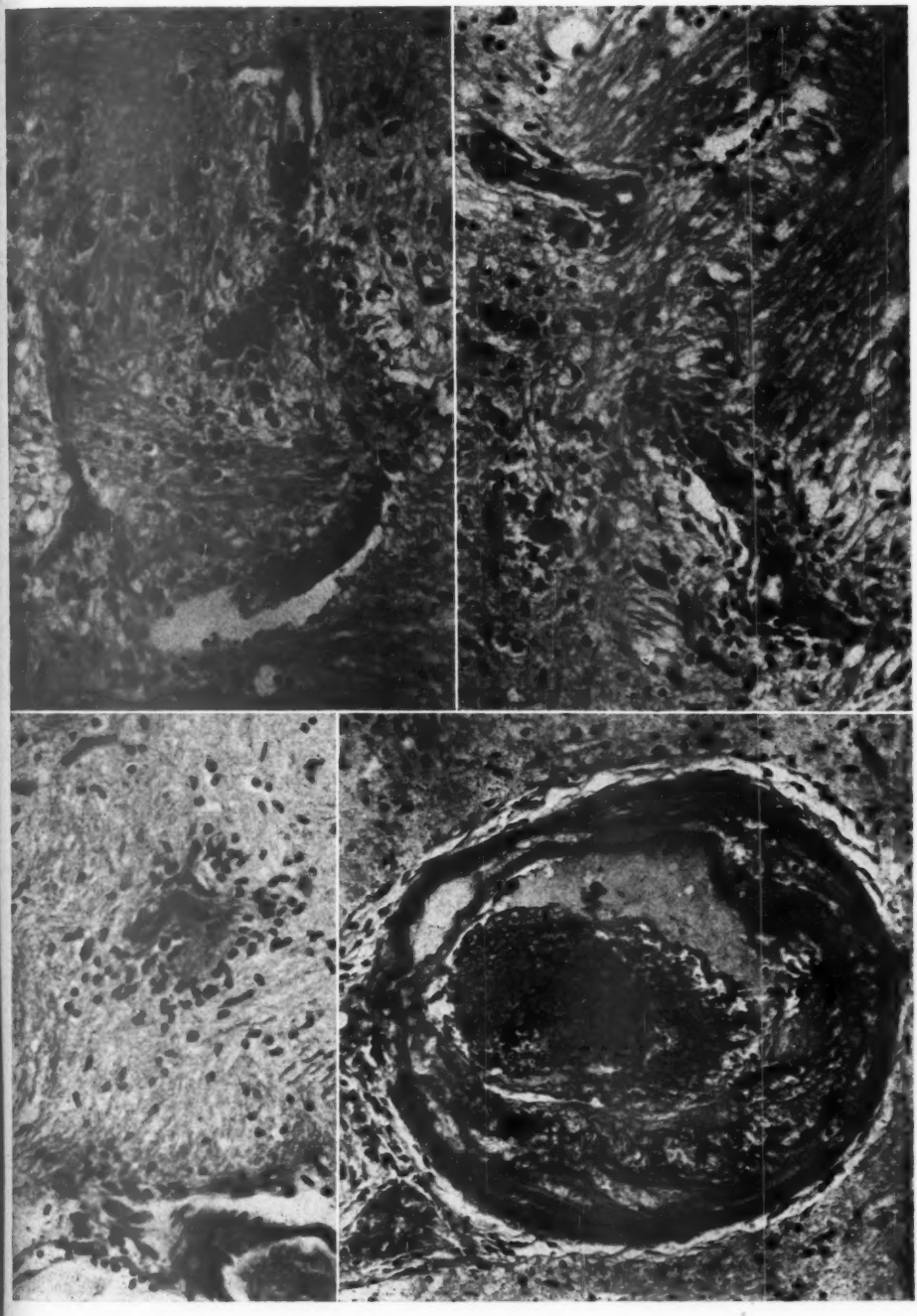
## DESCRIPTION OF PLATES

### PLATE 48

FIGS. 1 and 2. Case 1. Extreme cellularity and degenerative alterations of the cerebral capillaries. Hematoxylin and eosin stain. × 220.

FIG. 3. Case 1. Structureless, hyalinized nodule composed of a central homogeneous mass surrounded by a ring of proliferated glial cells and fibroblasts. Hematoxylin and eosin stain. × 220.

FIG. 4. Case 1. Disintegration and necrosis of a cerebral vein. Of note is the transformation of the vascular wall into a homogeneous substance devoid of either muscular or elastic elements. Hematoxylin and eosin stain. × 185.



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Scheinker Cerebral Capillaries in Arterial Hypertension

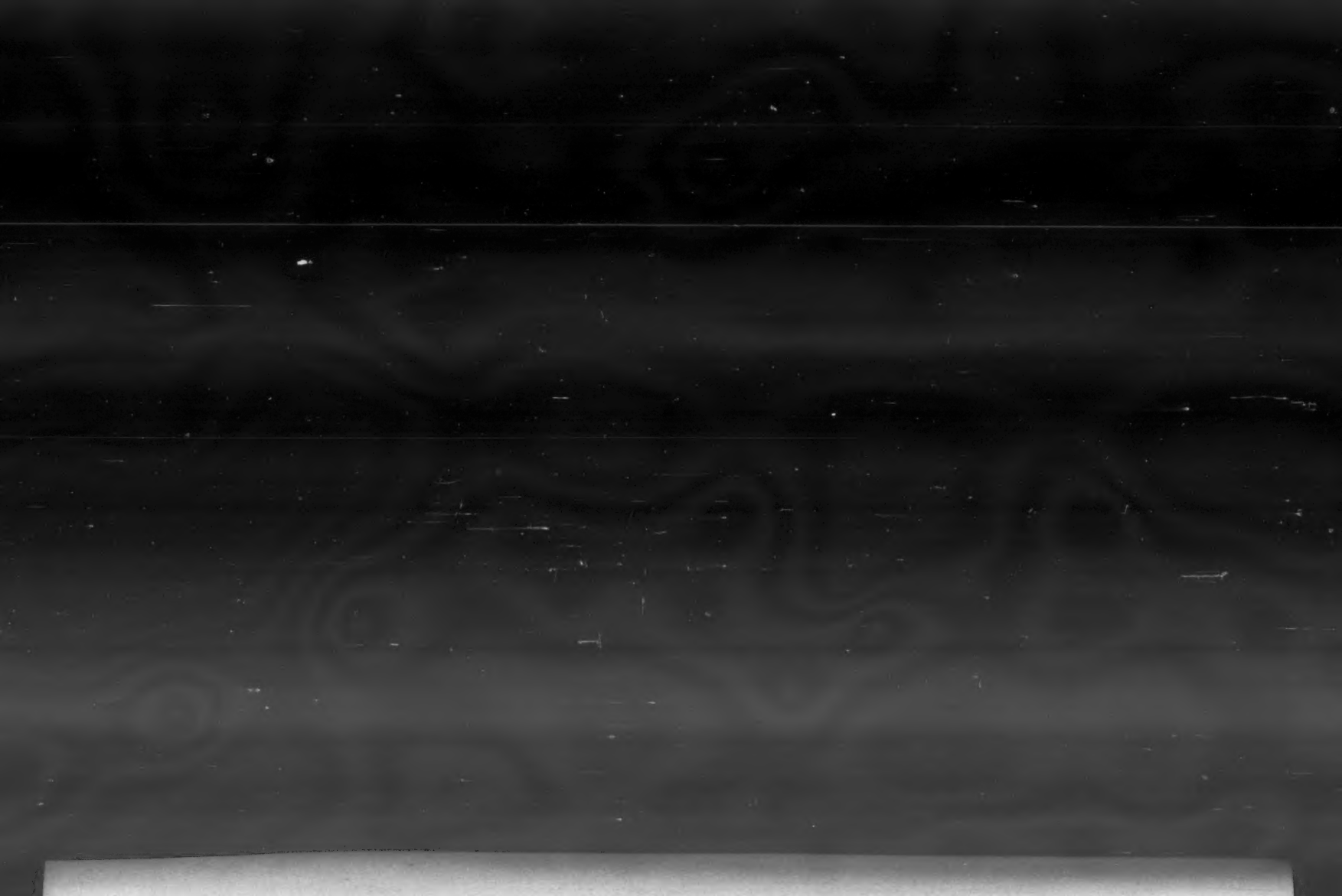
PLATE 49

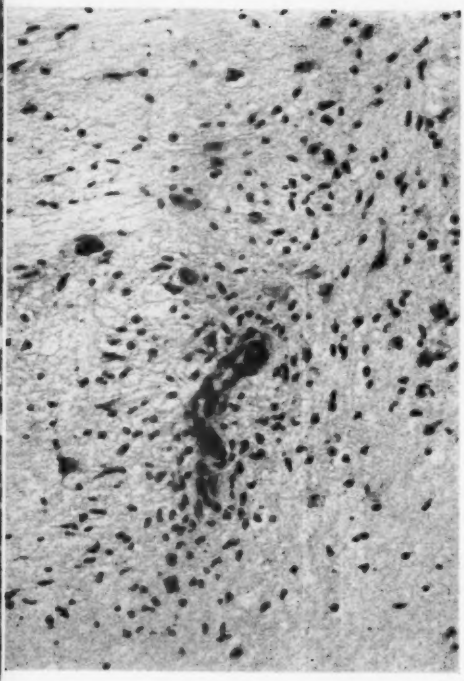
FIG. 5. Case 1. Spongy appearance of the white matter caused by numerous large oval spaces filled with serous fluid and by distention of the perivascular and pericellular spaces. Hematoxylin and eosin stain. × 185.

FIG. 6. Case 2. Increased cellularity of the cerebral capillaries caused by fibroblastic proliferation of the adventitial membrane and by hyperplasia and hypertrophy of the lining endothelium. Hematoxylin and eosin stain. × 220.

FIG. 7. Case 2. Homogenization and thickening of the capillary wall with resultant narrowing of the vascular lumen. Cresyl violet stain. × 220.

FIG. 8. Case 2. Arteriolar changes characteristic of hypertensive arteriolopathy. Of note is the extreme narrowing of the arteriolar lumen. Cresyl violet stain. × 220.





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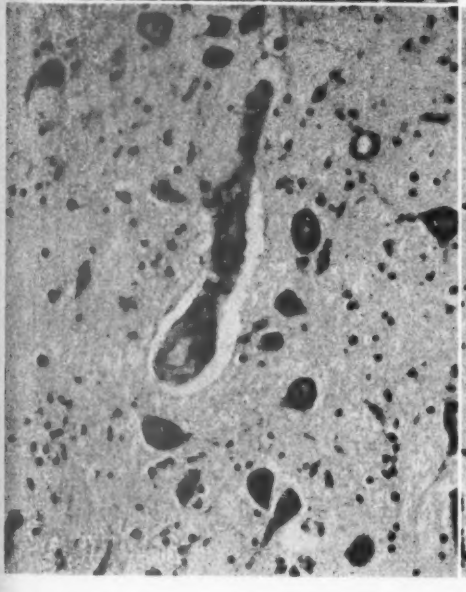

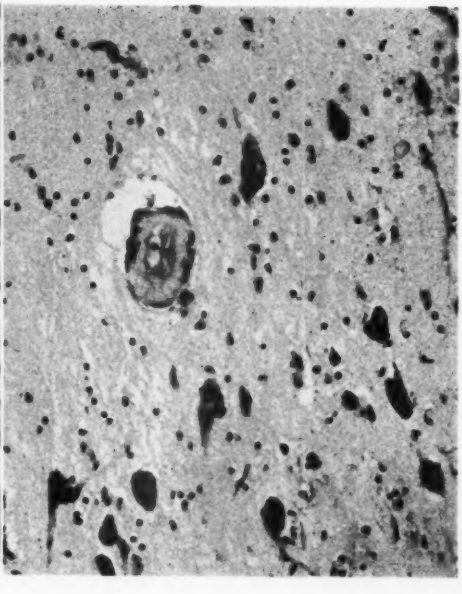

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Scheinker

Cerebral Capillaries in Arterial Hypertension

# COCCIDIOIDOMYCOSIS IN ANIMALS WITH REPORT OF A NEW CASE IN A DOG *

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The disease now known as coccidioidomycosis, or coccidioidal granuloma, was first encountered by Posada[1-3] in a human patient in Argentina in 1892; the same case was reported also by Wernicke.[4] However, it has remained for the southwestern United States to supply the great majority of the several hundreds of cases in the world's literature. Most of these have come from southern California, particularly the San Joaquin Valley, but the disease has been reported (in human beings) also from the states of Washington, Arizona, New Mexico, Texas, Louisiana, Colorado, Kansas, Nebraska, Missouri, Illinois, Tennessee, South Carolina, and Pennsylvania, as well as from Alaska, Hawaii, and Italy.[5,6] Very recently the disease has been reported from Army hospitals in a number of other states, but, owing to the constant movement of Army personnel, such cases were not necessarily indigenous to the areas from which they were reported.[7]

There is reason to believe that spores inhaled with dust in the desert countries of the Southwest are responsible for the more or less endemic status of the disease in these regions. It has been supposed that the causative fungus is capable of propagating on some native plant. On the other hand, Ashburn and Emmons[8] trapped 105 rodents in the desert near San Carlos, Arizona, and found gross pulmonary lesions of coccidioidal granuloma in 9 of them (7 pocket mice, 1 kangaroo rat, 1 ground squirrel), suggesting that these creatures may act as "reservoirs of infection." Direct spread from diseased to healthy subjects does not occur, it is believed, either among man or animals.

Two forms of this infection are now recognized in man, one of them being a benign acute respiratory disorder sometimes known as valley fever. The other form is the classical coccidioidal granuloma, a chronic progressive affection characterized by widely disseminated foci of a specific granulation tissue around the causative organisms and having clinically and pathologically a close similarity to tuberculosis. It is only this granulomatous type that has been recognized in other animals than man.

The causative organism appears in the tissues of its host as a refractile, doubly contoured spherule, from 5 to 50 $\mu$ in diameter; the name *Coccidioides immitis* bespeaks its striking resemblance to a coccidial

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oocyst. In the process of multiplication the enlarged parent spherule, looked upon by systematists as an ascus,[9,10] becomes filled with small endospores (ascospores) which, at maturity, are released into the surrounding tissue. When transplanted to the surface of common bacteriologic media the organism readily develops an extensive mycelium, producing a white, fluffy colony. According to Dodge,[9] there is no differentiation to form conidia.

Among animals, coccidioidal granuloma has been found principally in cattle. Giltner[11] reported the first case in 1918. In involved the bronchial and mediastinal lymph nodes of a bovine slaughtered at San Diego, California. He reproduced the disease in guinea-pigs and dogs, the inoculation of infectious material resulting in death within 2 months. About 70 additional cases in cattle have been brought to light in several reports by Stiles and co-workers[12-16] between 1933 and 1942. Beck, Traum, and Harrington[17] and Traum and Schalm[18] have reported approximately 20 cases. Their diagnoses were based upon demonstration of typical organisms in sections or cultures, or in both.

Findings in cattle may be summarized as follows: All cases have been from California, Arizona, or New Mexico with the exception of one from northern Colorado.[12] There were no observable clinical symptoms, the condition having been discovered in every instance in the course of routine veterinary inspection of carcasses slaughtered for food. Involvement was never very extensive, lesions being limited to bronchial and mediastinal lymph nodes, and, in a minority of instances, to small nodules in the lungs. The various descriptions of the lesions can be well represented by the words which Giltner[11] applied to his case: "Large areas of suppuration or several smaller purulent foci, all of which are usually surrounded by considerable granulation tissue and a fibrous capsule. Upon incising an affected gland there may be squeezed out a thick yellowish and tenacious pus." Several authors have noted the similarity of an incised lesion to that found in actinomycosis.

Davis, Stiles, and McGregor[14] described the histopathologic features as "granulomatous foci consisting of connective tissue, numerous blood-vessels, lymphocytes, plasma cells, mononuclear and polymorphonuclear leukocytes, a few eosinophiles, and many giant cells. The foci were surrounded by a connective-tissue capsule. Scattered throughout the inflammatory areas were many double-contoured spherical bodies, the majority of which had been taken up by giant cells. Scattered purulent areas containing spherical forms were seen within the granulation areas . . . . In some fields the inflammatory reaction resembled tubercle formations and only the presence of spherical bodies within the giant cells enabled a differentiation from a tuberculous process."

A striking feature of several of the bovine cases has been the presence of a "halo" of eosinophilic radiating "clubs" surrounding some of the Coccidioides spherules, entirely comparable to the "rosettes" of actinomycosis (as caused by *Actinomyces bovis*, also known as *Streptothrix israeli*) and actinobacillosis (caused by *Actinobacillus lignièresi*). This phenomenon has been described and illustrated by Davis, Stiles, and McGregor[14] and by Traum and Schalm.[18] Somewhat less pronounced manifestations of the same process have been seen in human tissues, as well as in experimentally inoculated guinea-pigs, with detailed studies by Moore.[19] But the occurrence of this poorly understood reaction around *Coccidioides immitis* is far from usual in any species of host, and it appears especially well developed in the specimens from bovines.

Cattle have been inoculated artificially. When large doses of the organism are injected subcutaneously the usual result is a well encapsulated abscess at the point of inoculation and rather limited involvement of the lymph nodes draining that region.

One case has been reported in a sheep from California by Beck.[20] The disease has been found in a mountain gorilla (*Gorilla beringeri*) and in an American monkey (*Cebus hypoleucus*), both in a zoo in San Diego, California, as reported by McKenney, Traum, and Bonestell.[21]

Coccidioidal granuloma has been found twice in the dog. The first case was reported by Farness[22,23] in 1940 and 1941 from Tucson, Arizona. The dog, a female Great Dane, 2 years old, was destroyed because of a gradually developing inability to use her hind legs, which was supposed to have been the result of severe rickets in early life. At necropsy, the lungs and, to a lesser extent, the liver, spleen, and kidneys, were found to contain numerous nodules resembling tubercles. Some of the nodules were described as necrotic. Microscopic sections made possible the diagnosis of coccidioidal granuloma, with demonstration of typical spherules. Since the brain and cord had not been examined, Farness pointed out the possibility that the inability to use the hind limbs might have been caused by a paralysis due to coccidioidal infection of the cord or its meninges, a type of involvement that is not rare among human cases.

The second instance of this disease in a dog was reported by Plummer[24] and by Radmore[25] in 1941. The animal was a male English Setter, 2 years old, domiciled at Hull South, Province of Quebec, Canada. The animal had never been away from that vicinity but had been mated with a female from California. Symptoms on first examination, as recorded by Plummer, included anorexia, emaciation, loss of activity, and reluctance to obey his master's call. Respiration and pulse were rapid; temperature was 103.2° F. (about 1° above the normal limit).

There was an occasional soft cough. Function of the bowels was normal and the feces were negative for parasitic ova. Urine was negative for albumin, sugar, casts, and cells. The red blood cell count, hemoglobin, and clotting time were within normal limits but there was a marked leukocytosis of 21,000 per cmm. The tuberculin test was negative. Within a week after the first examination the dog was much worse, showing symptoms of disturbance of the central nervous system. His eyelids drooped. He circled to the right, bumped into objects, refused food, and had spells of vomiting and polydipsia. He would roam aimlessly, usually at a walking pace, and, while so engaged, was struck and killed by an automobile 1 month subsequent to the first examination.

Significant lesions were found in the lungs and brain. The former were described (by Plummer [24]) as mottled, firm, and somewhat friable. Resemblance to tuberculosis was noted both grossly and microscopically, although acid-fast organisms were absent. Partaking in the inflammatory reaction were endothelial cells, lymphocytes, plasma cells, a few polymorphonuclear leukocytes, and numerous giant cells. Coccidioidal spherules were present in great numbers, sometimes free but usually within giant or endothelial cells.

The dog's brain showed an olive-shaped nodule, 2 by 1.4 cm., light in color and firm in texture, which was thought to be a glioma. (For this reason no bacteriologic studies were made.) Microscopically, the nodule consisted of epithelioid cells, with fibrous tissue in the deeper parts as well as round cells and a few polymorphonuclear cells. Coccidioidal spherules were present, although less numerous than in the lungs. They were diagnosed by several consultants as Coccidioides on the basis of structure and the absence of budding, for which a careful search was made. The third case of coccidioidal granuloma in a dog is reported in this paper.

## Report of Case

The subject was a female Fox Terrier, 7 years old in the spring of 1946. History supplied by the owner was to the effect that the dog was a native of central Iowa but had been living with her owner at an airfield at Eagle Pass, Texas, from the autumn of 1943 to the spring of 1945. During her stay there she was twice under the care of a veterinarian for an ailment which was diagnosed as a throat infection, and which caused dysphagia and loss of appetite. The animal's general health began to decline about 3 months after her return to Iowa, which was 8 months before her death in March, 1946.

The signs and symptoms upon hospitalization were doubtless attributable principally to nephritis but will be summarized briefly as follows: Stiffness and trembling with evidence of abdominal pain; inappetence, vomiting, polydipsia, frequent urination; pulse and respiration, accelerated; temperature, normal; red blood cells, 4,000,000 per cmm.; white blood cells, 24,000, of which 86 per cent were segmented polymorphonuclear leukocytes; hemoglobin, 9.64 gm. per 100 cc. (normal,

about 14 gm.); blood urea, 120 mg. per 100 cc. (normal, 10 to 20 mg.). Urinary findings were: pH, 6.0; specific gravity, 1.010; albumin, 3 plus, with large numbers of waxy and granular casts; negative for sugar, blood, acetone, bile salts, and indican. Cultures of the urine showed *Staphylococcus aureus* and *Streptococcus fecalis*. Fecal examination showed a few ova of *Ancylostoma caninum*. The animal was treated for about 2 weeks, a course of penicillin being included, but the white blood cell count rose to 25,000, and hematuria, a purulent vulvar discharge, and evidence of severe irritation in the urinary tract developed. Consequently, euthanasia was performed by electrocution.

At post-mortem examination a severe chronic purulent nephritis was found as anticipated, but the liver, lungs, and spleen presented lesions unanticipated from the clinical picture. Widely scattered through the liver were several conspicuous and sharply demarcated nodules of white or slightly gray tissue. They were irregular in outline, with a maximum diameter of about 1.5 cm. They were firm and dense to the touch, giving the impression of connective tissue. No pus or other material could be squeezed out of the cut surface. These areas were always in close relation to the larger bile ducts and blood vessels; at autopsy they were suspected of being manifestations of a neoplastic change in the walls of the bile ducts. There were three similar structures in the lungs, of no significant shape or location, and the spleen contained one nodule, almost perfectly spherical and about 6 mm. in diameter.

Smears from nodules in the liver were negative for acid-fast bacteria. Beyond this, no bacteriologic study was attempted.

Microscopic sections revealed the granulomatous nature of the lesions. The predominant cell was reticulo-endothelial in type, large, usually rather well rounded, and with a very generous amount of acidophilic cytoplasm. The nuclei were pale and vesicular, often indented, and more or less eccentrically placed. Macrophagic activity was evidenced by the rather frequent occurrence of lymphocytes and polymorphonuclear leukocytes within these cells. Polymorphonuclear neutrophils and lymphocytes also were important elements in the reactive tissue. Very commonly all three types of cells were intimately and rather uniformly intermingled, but in other areas there was a tendency for the polymorphonuclear cells to be gathered together to form tiny purulent foci. There were no giant cells and no necrosis or calcification. Stroma, or connective tissue elements, were minimal and inconspicuous, although small, well filled blood vessels were rather numerous. Sharp boundaries separated the granulation tissue from the normal structures which it was invading, but encapsulation, as a rule, did not occur. In the lung there were some areas where the new tissue was almost entirely fibrous, but even here it did not encircle the reticulo-endothelial areas.

Occasionally there was seen a spherule in one stage or another of the

developmental cycle of *Coccidioides immitis*. These were not numerous; most sections contained several spherules, but, of 129 sections examined carefully, 24 showed none. The forms most frequently encountered were simple spheres with a thick, refractile, doubly contoured wall and indistinct, amorphous contents. These were approximately 26 to 28 $\mu$ in diameter. More rarely a spherule was found in which endospores (ascospores) were formed or in the process of formation. These had thinner outer walls and varied from 35 to 40 $\mu$ in diameter. The first endospores to develop always formed a circle just inside the outer wall; in an equatorial plane about a dozen endospores made up this circle. At a more advanced stage additional endospores filled the center, so that some maternal spherules (asci) were seen completely filled with endospores. At what was doubtless a later stage, the outer cell wall had disappeared and the endospores were beginning to scatter. This scattering was observed to take place at two or three places around the circumference, suggesting that the outer wall had simply dissolved, although the usual conception seems to be that it bursts. Representative of a somewhat later period were irregular clumps of small, thin-walled spherules, each measuring about 6 $\mu$ in diameter, recently launched on their new existence as separate individuals. How many were successful in colonizing distant areas of the host's tissues is uncertain, but the fact that the larger forms are usually single leads to the surmise that the process of dissemination takes them some distance from their "birthplace," and, perhaps, that they suffer considerable mortality in this phase of their existence.

## Comment

It seems possible only to speculate on the route by which the infecting organisms gained entrance to the host's tissues. The respiratory tract is commonly incriminated in this respect and it may be that the pulmonary infection, in spite of its limited extent, was the primary site, with hematogenous spread to the liver and spleen. Opposed to this view is the much more extensive involvement of the liver. The graulomatous areas in that organ were not only more numerous but larger. To interpret the tendency of the hepatic lesions to be centered around large vessels and ducts as indicative of an ascending biliary infection is hardly compatible with the known propensities of this organism. But it points as much toward entry through the portal circulation as through the hepatic and general arterial system. On the other hand, at least some degree of access to the general arterial circulation is necessary to explain the single metastasis found in the spleen.

In view of the usually restricted geographic distribution of coc-

cidioidal granuloma, it is easy to believe that this dog acquired the infection during its stay in Texas. If such is the case the disease required a period of between 1 and 2½ years to attain the condition seen at necropsy. It should be noted, however, that the infection has been reported from the states which border Iowa on the west, south, and east. Sooner or later the first case could be expected to arise in Iowa; perhaps this is it.

Still more difficult to reconcile with the usual geographic distribution is the case of Plummer [24] and Radmore.[25] Either their dog picked up the infection in the Province of Quebec or else it acquired the disease by contact with the California dog (supposedly healthy) with which it was mated.

## SUMMARY

Nearly 100 cases of coccidioidal granuloma in cattle have been recorded, one in a sheep, two in captive wild animals, and two in dogs. A third case in a dog is described. This animal lived in Iowa, but there was a possibility that infection was acquired in Texas.

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## DESCRIPTION OF PLATES

### PLATE 50

FIG. 1. External surface of dog's liver, from the new case reported.

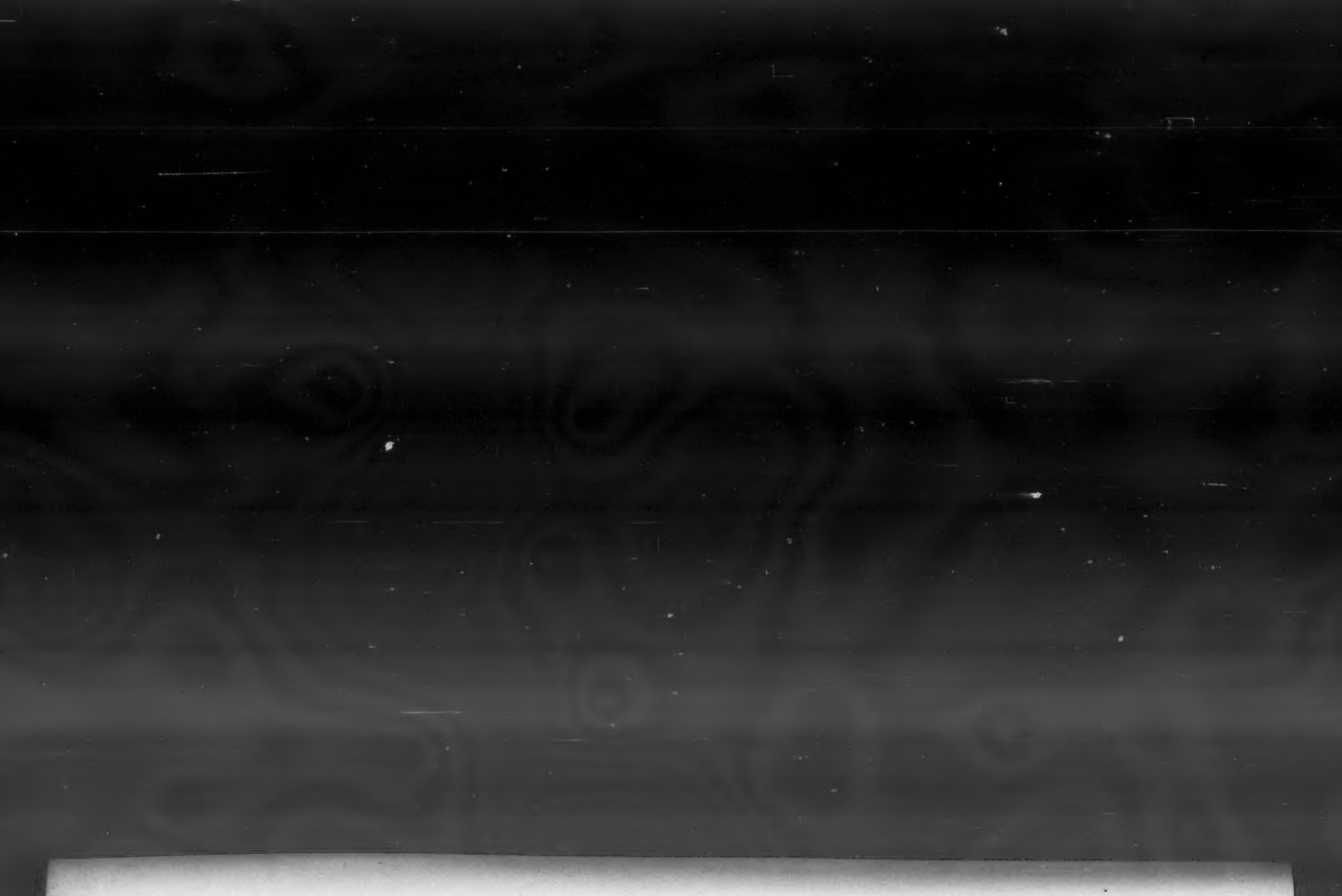
FIG. 2. Cut surface from the same liver. The white areas are granulomatous.

FIG. 3. The Coccidioides spherule in the simple, nonsporulating stage. × 620.

FIG. 4. Endospores are developing just inside the outer wall. × 620.

FIG. 5. Endospores have formed only subperipherally, but dissolution and dissemination appear to be taking place. × 620.

FIG. 6. Endosporulation is nearly complete. Polymorphonuclear leukocytes and reticulo-endothelial granulation tissue are seen. × 620.



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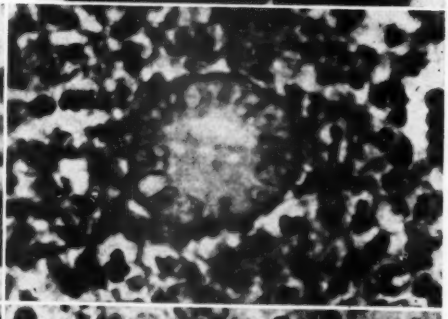

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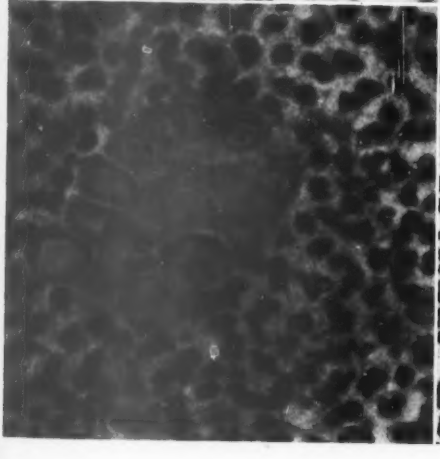

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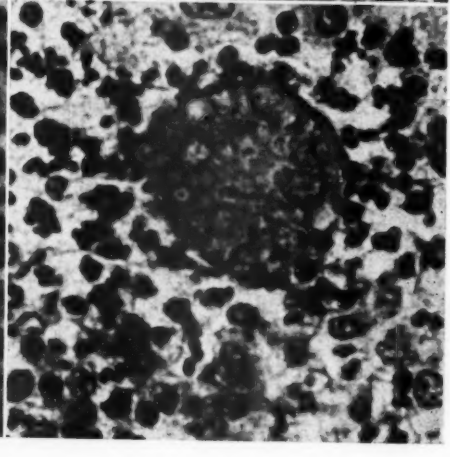

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PLATE 51

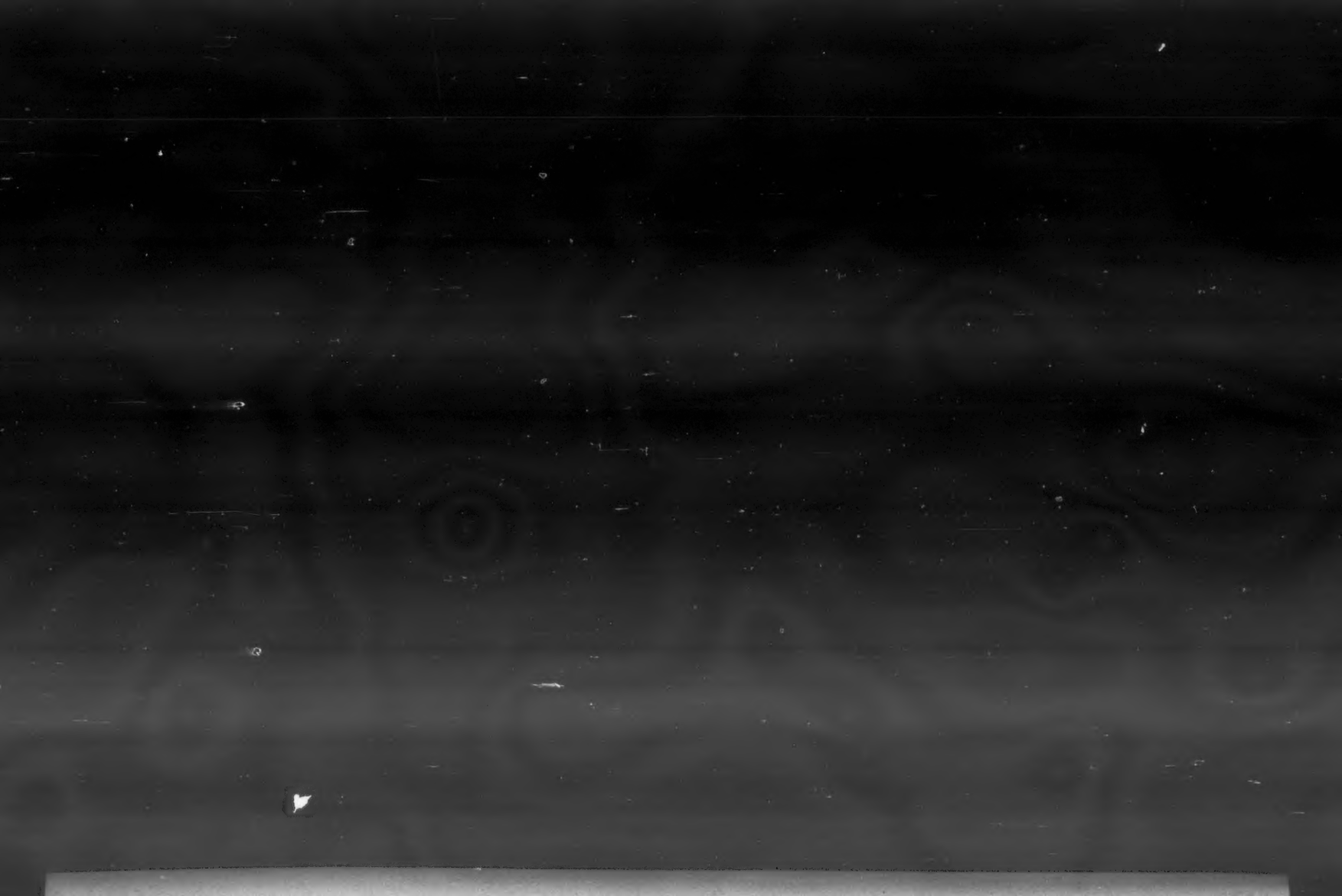
FIG. 7. Endosporulation is complete. × 450.

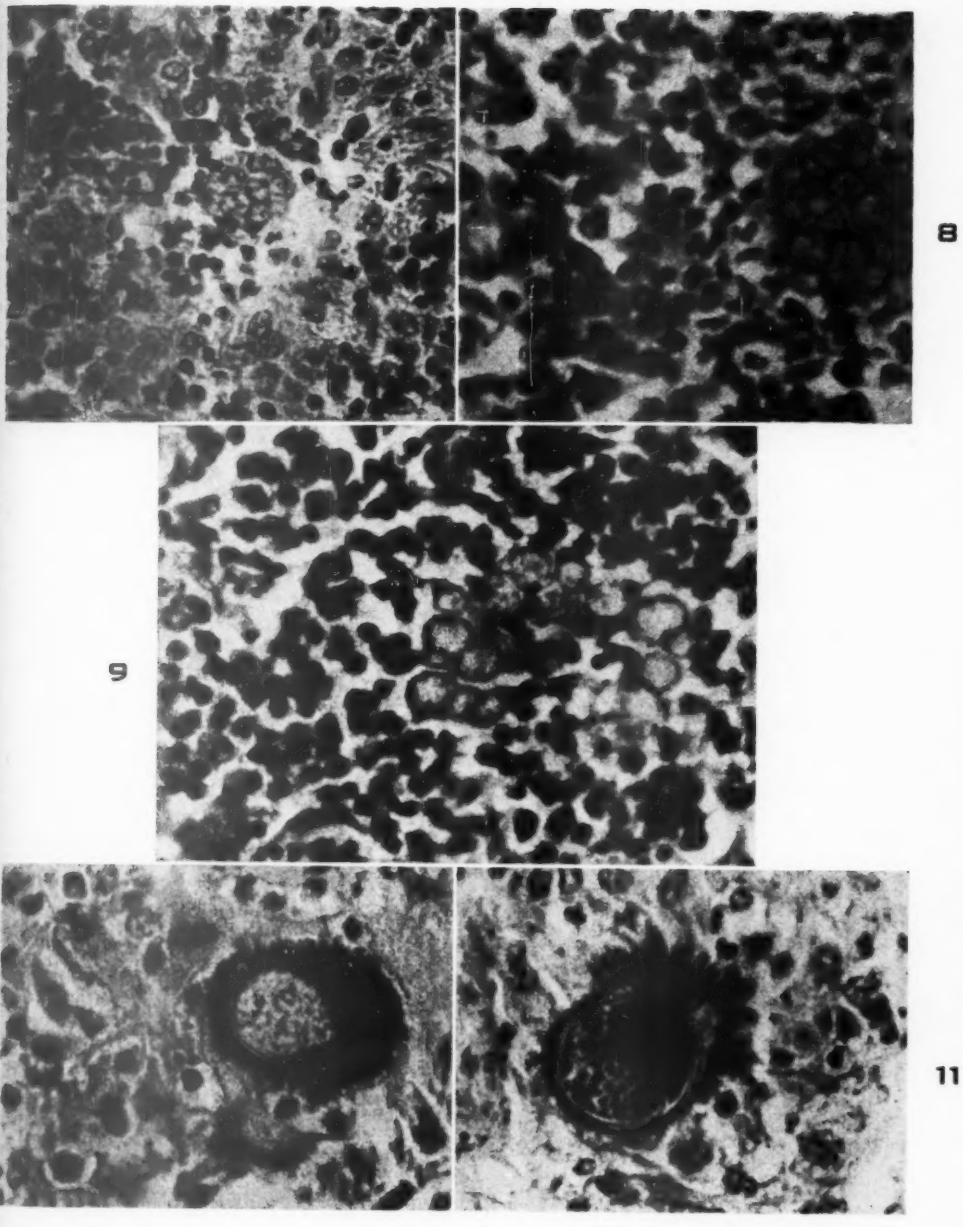
FIG. 8. The outer wall has dissolved, releasing the endospores. × 620.

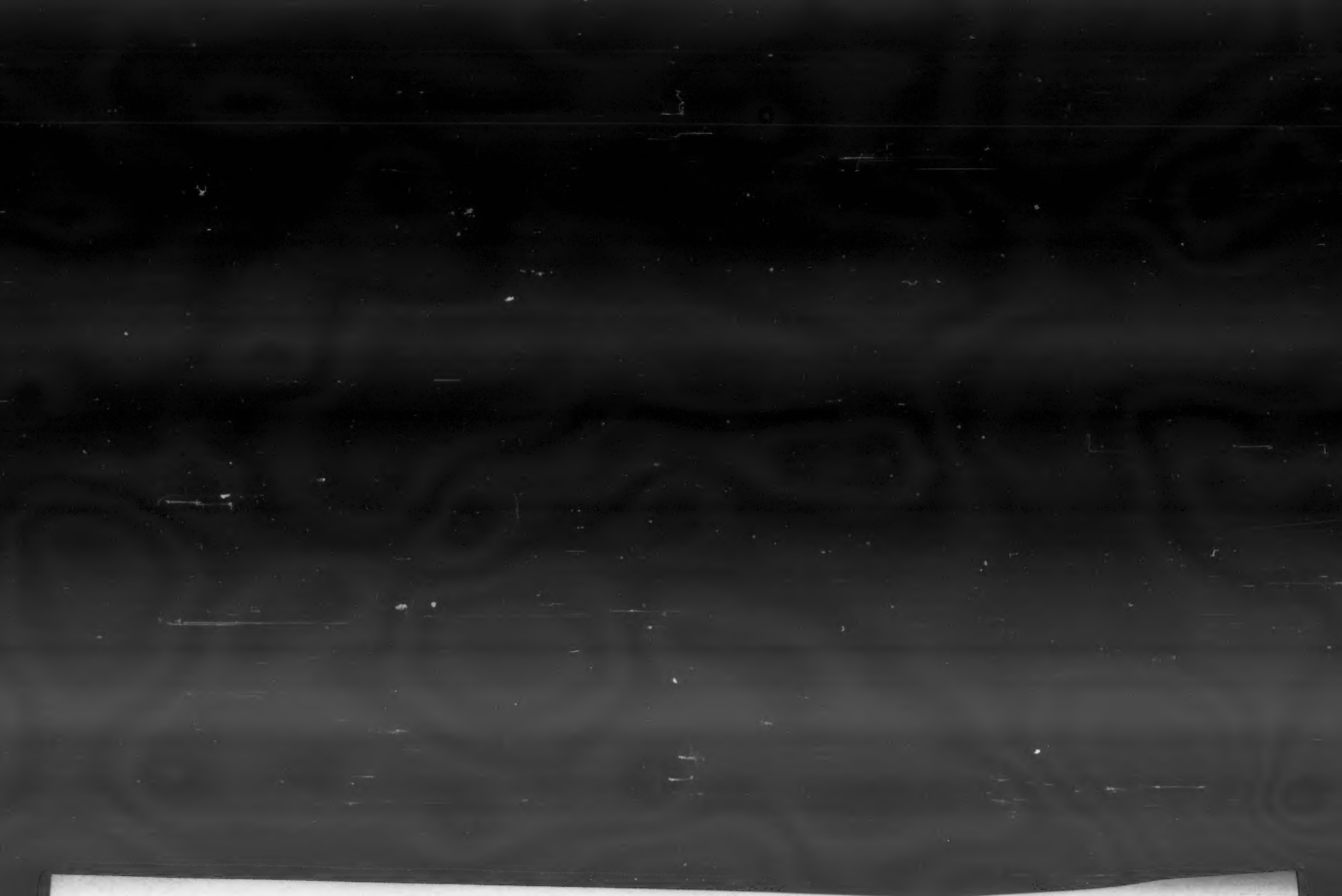
FIG. 9. Endospores are beginning to scatter. × 620.

FIG. 10. Rosette-like "clubs" surrounding *Coccidioides immitis*. These clubs stain bright red with hematoxylin and eosin. From a bovine case. (Tissue through the courtesy of Dr. C. L. Davis, Pathological Laboratory, U.S. Bureau of Animal Industry, Denver, Colorado.) × 620.

FIG. 11. Another rosette-like formation of "clubs," only partially encircling the Coccidioides organism. (Bovine case from Dr. C. L. Davis.) × 620.



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